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EVALUATION OF MICROBIAL DIVERSITY IN WETLANDS THROUGH POLYMERASE CHAIN REACTION (PCR) AND RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

Gregory K. Joseph, Captain, USMC

June 2006

DEPARTMENT OF THE AIR FORCE
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Abstract

The diversity of microbial communities in wetlands has not been fully measured. These communities may offer tools to naturally remediate sites polluted with chlorinated compounds, i.e., perchloroethylene (PCE). Restriction Fragment Length Polymorphism (RFLP) is a genomic characterization technique that is capable of rapidly characterizing bacterial communities and can provide valuable information about the diversity of microbial communities. By understanding the bacterial community researchers may be able to enhance natural attenuation of polluted sites. Soil samples were collected from a constructed wetland receiving PCE-contaminated ground water at Wright-Patterson Air Force Base (WPAFB) in Dayton Ohio and a naturally-occurring and non-polluted wetland in Beavercreek, Ohio. Samples were taken seasonally (winter, spring, summer, and fall), and at three depths (0 to 9, 9 to 27 and 27 to 45 inches) and RFLP patterns were within and against wetlands. Both sites were similar except for bands of 1400, 1000, 850, 550, and 300bp. For the depth, differences of 550 and 300bp were seen between wetlands. Seasonal differences of 1400, 1000, 850 and 300bp were also seen at both sites. Specific cores and sections i.e., winter sample section 2 and fall section 3 (treated wetland), as well as spring section 3 and summer section 2 (non- polluted wetland) unique bands emerged. For overall seasonal band diversity, the annual base pair total of both wetlands was compared by dividing it by each total seasonal band size giving the following: fall 96.6%, winter 62.5%, spring 56.25%, and summer 69.32% suggesting that optimal conditions for relative band diversity is seen in fall perhaps due to moderate temperatures or even microbial community reaching balance within each other. As temperature decreases (winter), bacterial more resistant to colder temperatures seems to increase band diversity. Band diversity comparison through all wetland depths was calculated as total base pair depth of both wetlands divided by total

individual wetland depth giving the following: 79.37% in the first depth, 100% in the second depth, and 79.37% in the last depth, suggesting perhaps that more substrate is present between 10 and 28 inches of all wetlands. Band diversity comparison between wetlands was calculated as total base pair size of both sites divided by total individual site giving the following: Valle Greene 100% band diversity and WPAFB 54.7%; this difference perhaps suggests a bacterial community that has out-competed the other communities for substrate availability, i.e., PCE thus optimizing its growth and forcing a reduced bacterial diversity.   Lastly, further analysis in-between sections and intra-seasons are needed as the bacterial profiles differ significantly in both wetlands.

Based on the results obtained, PCR-RFLP seems to be a reliable procedure for the routine description of bacterial communities in wetlands.

Acknowledgements

I would like to express my sincere appreciation to my faculty advisor, for his guidance and support throughout the course of this thesis effort. His patience, insight, and experience were invaluable to my efforts.

I would, also like to thank my Wright State University advisors for their total involvement throughout this effort. Their knowledge of wetlands and molecular concepts assisted me immeasurably through my research and was instrumental in the success of my thesis; without your guidance my research would have never come to completion.
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To my wife and children; without your support, patience, and understanding this endeavor would not have been possible. You committed yourself to me without question or reservation allowing me to focus and accomplish this personal goal. I will forever be in debt to you.

Above all, my endurance and motivation were a direct reflection of the mercy that God has for his children; He gave me clarity and guided my path when deadlines and projects seem impossible to accomplish. "I can do all things through God who strengthens me", Philippians 4:13

Gregory K. Joseph

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EVALUATION OF MICROBIAL DIVERSITY IN WETLANDS THROUGH POLYMERASE CHAIN REACTION (PCR) ANALYSIS AND RESTRICTION FRAGMENTED LENGTH POLYMORPHISM (RFLP)

I. Introduction

1.1 Overview

Chlorinated solvents were used in industrial cleaning and degreasing processes in the United States since the early 1900s and their production increased significantly with the growth of industrial processes over the past century (Pankow, 1996). The uncontrolled release of these solvents has created environmental concerns. The high cost of physically removing chlorinated solvents from water sources has led to the pursuit of less expensive options, and in-situ bioremediation methods have shown good results (BonDurant). Microbiological processes at work in groundwater systems have the ability to break down chlorinated solvents such as PCE into harmless end products like chloride ions, carbon dioxide (CO_2) and water (Lee et al., 1998). The isolation and identification of these degrading microorganisms has been difficult and expensive. A current method of identifying bacteria without the need to cultures relies on using direct amplification of bacterial 16S rRNA extracted from soil. Result can be compared to qualitative culture-based method. The time requirements of this approach, coupled with the inability to accurately characterize a bacterial community under field conditions has forced researchers to seek alternative solutions. Therefore, rapid analysis of diversity in complex microbial communities has become an important goal in microbial ecology (Dunbar, 2001). Community diversity can be examined at several levels. The most simple analyses use DNA profiles (generated by RFLP) to identify differences in the composition of communities (Lorah, 2003). More refined approaches describe differences not only in community composition, but also in community organization, by

measuring the number (richness) and relative abundance (structure or evenness) of species or phylotypes (Lorah, 2003). Richness and evenness of biological communities reflect selective pressures that shape diversity within communities. Measuring these can be useful when assessing treatment effects (e.g., physical disturbance, pollution, nutrient addition, predation, climate change, etc.) on community diversity.

Restriction Fragment Length Polymorphism (RFLP) is a culture-independent method of obtaining a genetic fingerprint of a microbial community (Blackwood, 2003). This procedure uses restriction endonuclease enzymes that cleave DNA molecules at specific nucleotide sequences, depending on the particular enzyme used. If DNA molecules differ in nucleotide sequence, fragments of different sizes can be seen through gel electrophoresis. These fragments can be used to compare to bacterial communities**.** In essence, RFLP is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of a particular restriction endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme (Edwards, 1999). In this study, the cleavage patterns generated will be used to differentiate bacterial groups from one another and between populations found in wetlands subjected to different conditions.

Background

Chlorinated solvents have been found to be carcinogens (U.S. Department of Health and Human Services, 2003), hence requiring removal from contaminated areas. Unfortunately, chlorinated solvents are persistent in soils and degrade slowly. This slow degradation requires significant man-hours spent on remediation that translates into potentially economic burdens and significant health hazards. Fortunately, certain microorganisms, (e.g., *Dehalococcoides* and *Desulfuromonas*) are believed to naturally degrade halogenated compounds (Lorah, 2003). Thus,

the employment of known dehalogenating microorganisms has surfaced as an effective procedure for remediation of polluted sites. Even though the potential capabilities of certain microbial consortia to degrade halogenated compound (perchloroethylene-PCE) have been postulated, the microbial intervention is inconsistent (Baker, 2003). Therefore, the employment of additional experimental procedures, leading to isolating microbial species, must be incorporated into community characterization. More specifically, the use of genomic techniques for the identification of microbial consortia i.e. Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphisms (RFLP), are the necessary techniques to accomplish this research.

Purpose of Research

The intent of this study is to analyze and compare microbial communities through RFLP analyses so that we may compare these communities in an undisturbed/natural wetland and a contaminated, man-made wetland.

Research Objective

The main objectives of this research are:

1. To examine the cleavage pattern within PCR-amplified 16s rRNA genes for differentiation of bacterial groups present in wetlands.
2. To identify bacterial community changes vertically and seasonally in the two wetlands
3. To compare bacterial communities between wetlands for similarities and establish these similarities as the building block for potential follow-on research in natural remediation.

Research Focus

This research focuses on using RFLP analysis to establish a fingerprint for the bacterial communities in an undisturbed/natural wetlands and a PCE contaminated man-made wetland.

Methodology

The first stage consisted of field work in which soil core samples were extracted from both wetlands. Stage two consisted of laboratory work where DNA extraction, PCR, and RFLP experiments occurred. Lastly, RFLP base pair profiles were evaluated and compare between sites for similarities and variability.

Research/Limitations

The following are limitations made during this research:

1. *Rsa*I was used in digestion thus creating a specific profile in all wetlands thus increasing potential bias in restriction sites.
2. Financial and time constraints only allowed for 1 core sample from each season, each location, and each depth thus increasing the effect sampling error of this data.
3. 0.25g of soil was used to represent the entire wetland therefore potentially not depicting the full range of the bacterial community in each wetland.

II. Literature Review

Overview

This chapter summarizes research into bacterial identification in wetlands that is pertinent to this study. Background topics include a historical perspective on PCE polluted sites, potential remediation techniques, description of wetlands to include its physical composition, importance in maintaining the ecosystem balance, and its ecosystem complexity specifically the microbial diversity. Following these sections, pre-established experimental procedures in microbial isolation/fingerprinting will be illustrated through genomic techniques (i.e. DNA extraction, PCR, and gel electrophoresis). RFLP techniques will be described as the procedure to rapidly identify microbial communities. Lastly, experimental data (microbial diversity) will be interpreted in order to validate bacterial community composition in wetlands.

Historical Perspective

Chlorinated solvents are among the most common groundwater contaminants in the United States. Of the 1,636 sites currently listed under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) National Priority List (NPL), volatile organic compounds (VOCs), to include PCE have the greatest frequency of occurrence (NRC, 1994; U.S. Department of Health and Human Services. ATSDR, 2003) (Table 1).

Table 1. Chlorinated VOCs Frequencies of Occurrence

NPL Ranking	Name	NPL Site Frequency
4	Vinyl chloride (VC)	608
16	Trichloroethylene (TCE)	1021
30	**Tetrachloroethylene (PCE)**	**930**
43	Carbon Tetrachloride	422
87	1,2-Dichloroethane	599
148	1,1,2,2-Tetrachloroethane	327
163	1,1,2-Trichloroethane	274
175	1,2-Dichloroethene, Trans-	598
213	1,2-Dichloroethylene	450
277	1,2-Dichloroethene, Cis-	263
282	Dichloroethylene (DCE)	114

Source: (U.S. Department of Health and Human Services. Agency for Toxic Substances and Disease Registry, 2003). Ranking based on combination of toxicity, frequency, and potential for human exposure.

PCE was produced in large quantities and widely used in a many operations including dry-cleaning of fabrics and metal degreasing operations (Chapelle, 2001). This study highlights the chlorinated aliphatic PCE (Figure 1) and the bacteria consortium associated with its degradation.

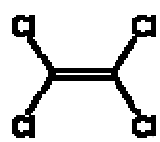


Fig 1. Tetrachloroethylene (PCE).

Around 1950 disposal of PCE was unregulated. Negligent disposal practices resulted in groundwater contamination due to this chemical's physical and chemical properties (U.S. EPA, 1988). This unsupervised disposal of PCE forced environmentalist to focus on ways to clean the polluted sites. The following summarizes the approaches in remediation of polluted sites.

Remediation Techniques- Natural Attenuation

The United States Environmental Protection Agency (USEPA) has defined natural attenuation as the naturally occurring in-situ processes that reduce the mass, toxicity, mobility, volume, or concentration of contaminants. Attenuation processes include biodegradation, dispersion, dilution, adsorption, volatilization, and chemical or biological stabilization or destruction of contaminants (USEPA, 1997). Natural attenuation relies on naturally occurring processes to degrade contaminants. Research indicates that, under the right conditions, PCE will degrade as a result of microbial activity into less chlorinated products and possibly even all the way to innocuous substances (Lee et al, 1998). However, the conditions needed to effectively remediate chlorinated contaminants such as PCE are rare in nature, and some intervention is often required. For example, a contaminated aquifer could be seeded with microorganisms that are known to decontaminate PCE, as well as a substrate could be added to serve as a source of carbon and energy for the microbes (Lorah, 2003).

Natural attenuation is typically reserved for sites where there is no immediate threat to the safety the local drinking water supply (Amon, 2005). Those sites that are found to be a threat to the supply require a more proactive approach. Contaminants such as perchloroethylene are often associated with dry cleaning operations or industrial sites where chlorinated solvents were used. These sites are often located in populated areas where the slow natural attenuation process is often deemed unacceptable by regulators and/or the public as a viable remediation strategy.

The natural wetland environment possesses the characteristics necessary for the complete degradation of chlorinated aliphatic hydrocarbons. While the deeper, anaerobic regions of the wetland foster transformation of more highly-chlorinated contaminants like PCE and TCE, the aerobic zones near the surface or around ports are conducive to the degradation of DCE and VC to

ethane and CO_2. This phenomenon has been observed in natural wetlands at the Aberdeen Proving Grounds in Maryland (Lorah and Olsen, 1999), and it has been replicated in constructed wetlands one built at WPAFB where this research was conducted.

In order to naturally remediate sites polluted with PCE, several procedures must be considered. The following summarizes possible techniques to naturally remediate polluted sites.

Phytoremediation

During this process, plants are used to remove, transfer, stabilize, or destroy contaminants in soil, sediment, and groundwater. The main steps in phytoremedation are: 1. enhanced rhizosphere biodegradation, 2. phytoextraction, 3. phytodegradation, and 4. phytostabilization (US EPA, 2001). Enhanced rhizosphere biodegradation takes place soil or in the groundwater that immediately surrounds the plant roots. Phytoextraction occurs when the plant roots uptake the contaminants and they are deposited in the plant shoots and leaves. Phytodegradation is the conversion of contaminants within plant tissues and phytostabilization is when plants produce chemical compounds to prevent the contaminants from dispersing and staying within of roots and soils. Figure 3 shows the basics of phytoremediation process.

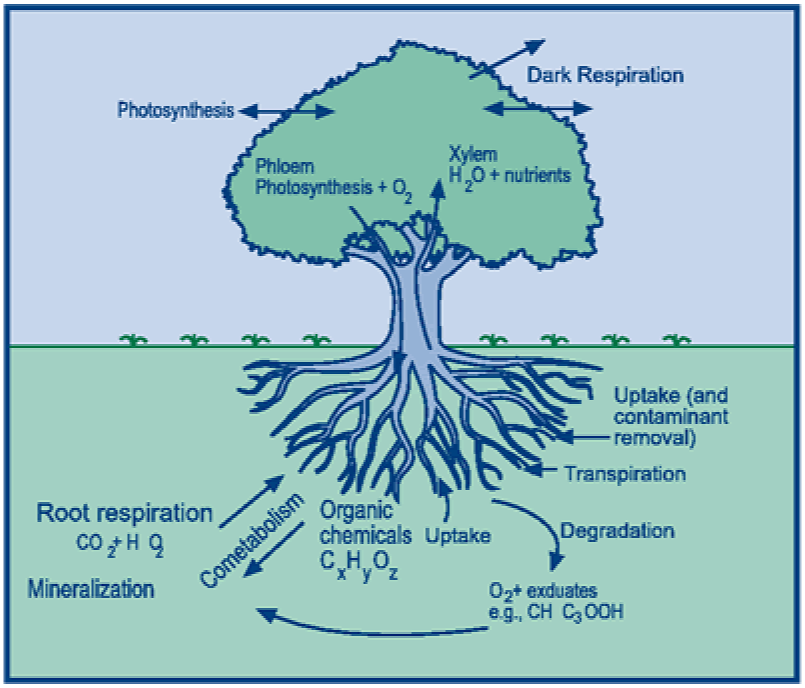


Fig 2. Processes in Phytoremediation (EPA Treatment Technologies for Site Cleanup, 2001)

<u>Bioremediation</u>

In bioremediation microorganism degrade organic contaminants in soil, sludge, and solids. The process can be done *ex situ or in situ*. The microorganisms use the contaminants as a food source (electron donor) or as a electron acceptor. *In situ* bioremediation is performed in place; this procedure stimulates and creates an environment where microorganisms can use contaminants as substrate. One of these processes is reductive dehalogenation, i.e., dechlorination of PCE. Figure 3 highlights the steps in reductive dechlorination.

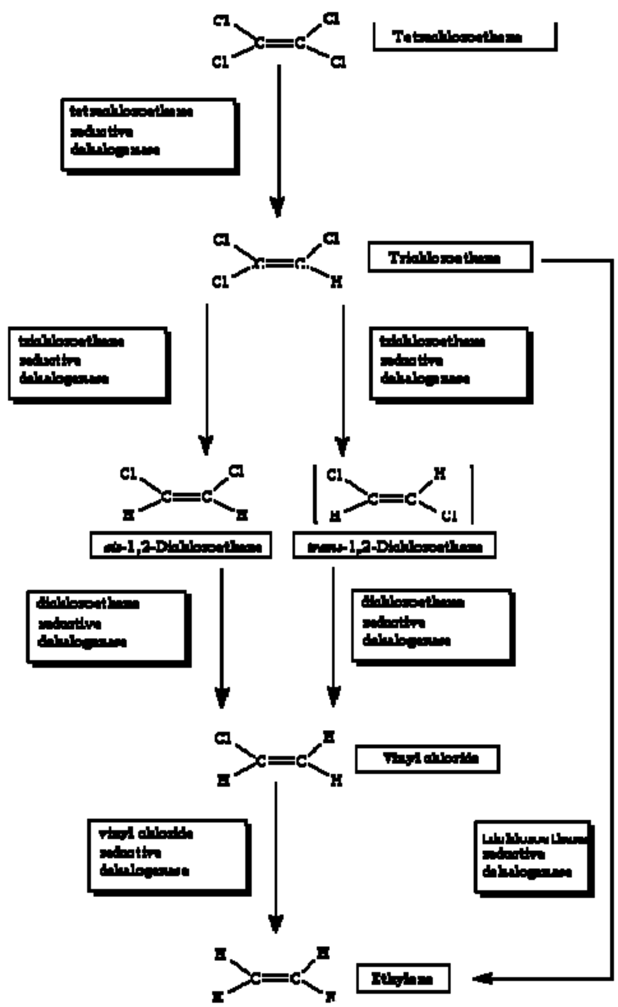


Fig 3. Reductive Dechlorination of PCE (Ellis and Anderson, 2003)

The processes of biodegradation of chlorinated aliphatic hydrocarbons in the natural environment is a oxidation-reduction (redox) reaction in which the chlorinated solvent acts as an electron acceptor and replaces a chlorine atom with a hydrogen atom (Lee et al., 1997). Reductive dechlorination removes chlorines from the contaminant but only occurs under anaerobic conditions. Chlorinated solvents may also act as electron donors providing an energy source for certain microorganisms. This occurs predominantly in aerobic conditions and is most often associated with the less-chlorinated solvents such as vinyl chloride (Fogel, 1995). With the continual exchange of electrons providing energy for microbes, oxidation-reduction (redox) chemistry is an important concept in chlorinated hydrocarbon transformation (Lee, 1998). PCE degradation generally requires reducing conditions suitable for methanogenesis before the organism will transform it to ethene (McCarty, 1996). Such conditions require the presence of enough organic substrate to reduce all of the oxygen, nitrate, iron, and sulfate before dechlorinating bacteria will successfully compete to reduce chlorinated aliphatic hydrocarbons (Lee, 1998).

Microbial populations face environmental changes including temperature, pH and availability of new substrate. Temperature dictates metabolic rates and completely halts metabolism in extreme cold. Conversely, as temperature increases, metabolism increases until reaching an optimum temperature level or "cardinal temperature" that is characteristic of a particular microbial strain (Lorah, 1999). While the effect of temperature on metabolism also depends on pH and salinity, most subsurface bacteria operate most efficiently at a temperature range of 20-30 degrees C. (Chapelle, 2001). In addition to temperature, pH affects microbial metabolism by dictating the aqueous solubility and complexing ability of groundwater. Low aqueous solubility caused by low pH levels results in an increase of lipid solubility that decreases

the bioavailability of organic acid substrates in ground water (Fogel, 1995). The rapid depletion of oxygen following the sudden influx of carbon substrates from contamination to an aerobic environment can lead to anaerobic conditions and anaerobic metabolism mechanisms involving the new electron donor substrates and new electron acceptors to go along with them. These new reducing conditions can lead to further microbial acclimation outcomes.

Description of Wetlands

According to the Environmental Protection Agency (EPA), wetlands are areas where water covers the soil, or is present either at or near the surface of the soil all year or for varying periods of time during the year. Additionally, "wetlands are defined as land where the water table is at (or above) the ground surface long enough each year to maintain saturated soil conditions and the growth of related vegetation" (Reed et al., 1995). Wetlands have the capacity to support both aquatic and terrestrial species. The prolonged presence of water creates conditions that favor the growth of specially adapted plants (hydrophytes) and promote the development of characteristic wetland (hydric) soils (Wetland Research, Program, 2003). Wetlands vary widely because of regional and local differences in soils, topography, climate, hydrology, water chemistry, and vegetation.

Natural Wetlands

Wetlands are complex ecosystems that perform numerous beneficial functions in nature from erosion control to nutrient sinks and sources. As depressions in the ground, some natural wetlands can buffer downstream locations from the effects of heavy rainfall events and reduce the potential for flooding (Wetlands Research Program, 1993). The damping effect of wetland vegetation also reduces the velocity of waters passing through the wetland; reducing the re-suspension of sediments, which improves water clarity and quality (Wetlands Research Program,

1993). It is this ability to purify surface and ground waters that makes wetlands a potential remediation technology. The high microbial activity and lack of oxygen diffusion often associated with wetland soils creates a situation where both aerobic and anaerobic zones are in close proximity to each other. This characteristic makes it possible to completely degrade resistant compounds like PCE which may require a series of reducing and oxidizing environments (Amon, 2005). Figure 4 illustrates the processes involved in degradation of halocarbons in wetlands.

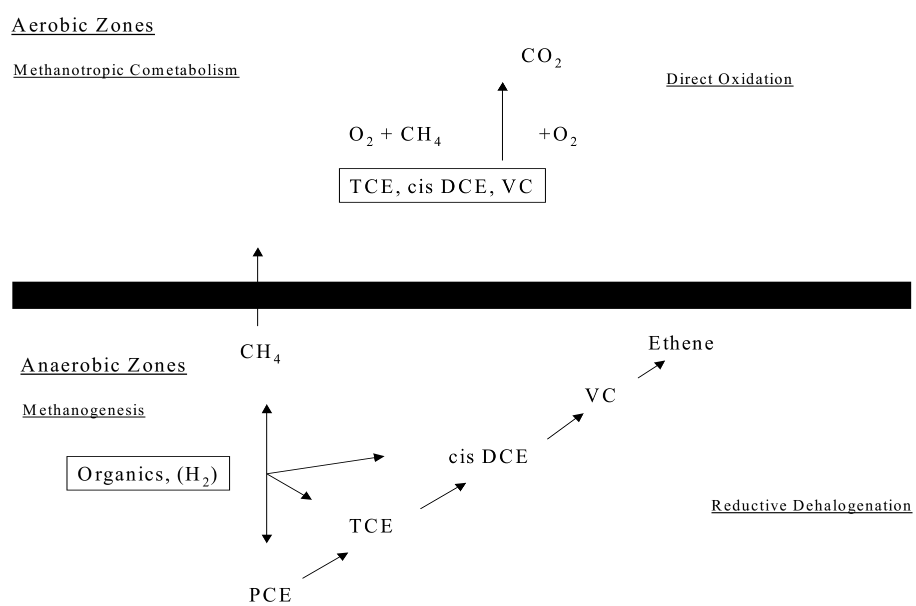


Fig 4. Cometabolic Oxidative Dehalogenation (Opperman, 2002)

Natural wetlands are considered Waters of the United States and therefore subject to regulation under the Clean Water Act. This act requires a permit for the addition or discharge of any pollutant from a point source into these protected waters, and such permits normally require treatment to a specified effluent standard before release into the environment (EPA, 1988). Regulations prevent the intentional use of naturally occurring wetlands for remediation. Figure 5 shows the location of naturally-occurring wetland located in Dayton, Ohio that was used in this research. It is approximately 700 acres and is characterized by its groundwater dominated

hydrology, high conductivity water, richly diverse sedge dominated community and absence of standing water.

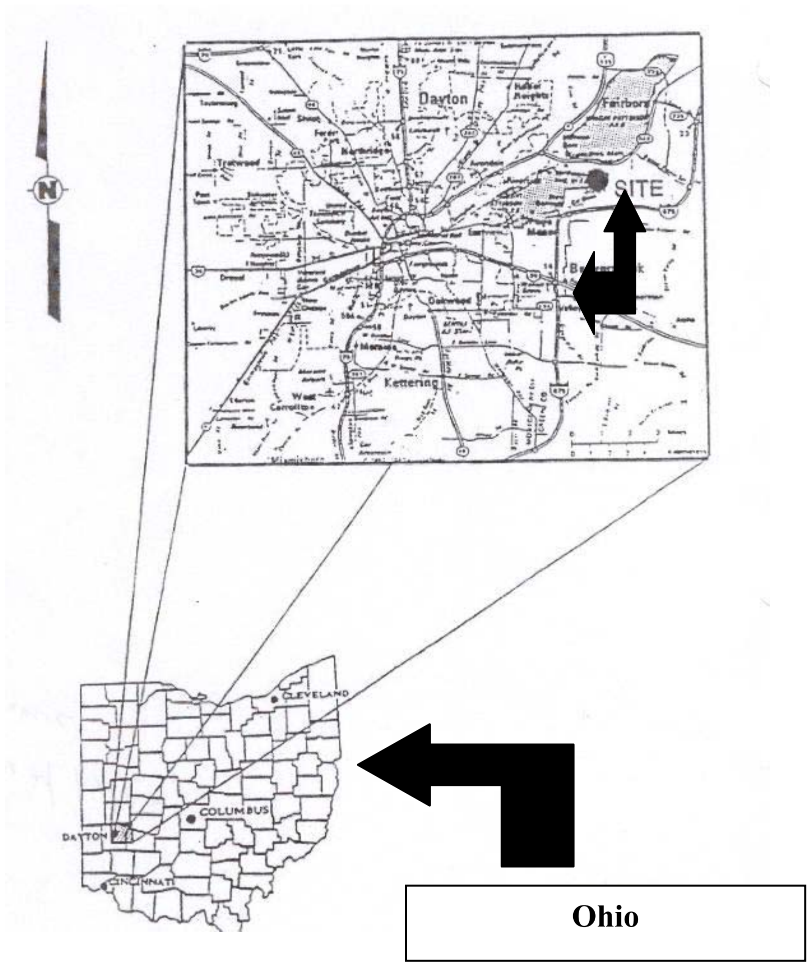


Fig 5. Valle Greene wetland

Constructed wetlands, on the other hand, are largely free from regulatory oversight and ecological concerns inherent with natural aquatic ecosystems (Reed et al., 1995). The following section will highlight the characteristics of constructed wetlands.

Constructed Wetlands

Constructed wetlands are used as an alternative in remediation of sites that are polluted with dangerous compounds, i.e., PCE (Amon, 2005). In this study, data was accumulated from a constructed wetland erected by Wright State University and the Air Force Institute of Technology (AFIT). This wetland is located in Dayton, Ohio at the WPAFB area C complex. This research employed the pre-exiting wetland up-flow design as a template for remediation processes. The design allows for contaminated groundwater to be pumped through the hydric strata of the wetland, first encountering the anaerobic region where PCE and TCE can be reduced, and then encountering the aerobic region where other chlorinated byproducts can be oxidized (Amon, 2005). The wetland cells are situated over an aquifer that is contaminated with a plume of PCE. The site has been identified and documented with the Ohio Environmental Protection Agency with no current requirement for remediation. The cells represent what is hoped to someday develop into a low cost, low energy, pump and treat system that relies on natural processes to attenuate PCE (Amon, 2005). The concept is supported by findings at a U.S. Department of Defense site in Aberdeen, Maryland, where a natural, groundwater-fed wetland appears to demonstrate the complete destruction of trichloroethylene - a related VOC and also a degradation product of PCE (Lorah and Olsen, 1999).The soil composition of the subsurface media differed slightly in the wetland. The cells contained 3 layers of hydric soil (i.e., soil characterized by anaerobic or reducing conditions when saturated), the bottom layer of which consisted of a mixture of approximately 10% woodchips added to the hydric soil to provide an initial source of available organic carbon to facilitate microbial growth. The second cell contained a middle layer of iron-rich soil sandwiched between two layers of hydric soil with no woodchips added. Table 2 depicts the composition of the soil at the treated wetland.

Layer	**Cell 1**
Top	Hydric Soil (likely root zone)
Middle	Hydric Soil
Bottom	Hydric Soil (organic matter added)

Table 2 Composition of the Soil Layer in treated wetland (Entingh, 2003)

Additionally, the roots of wetland vegetation planted in the top layer of both cells were believed to permeate all three layers, providing oxygen to sustain different aerobic reactions in the root zone (Amon, 2005). When conditions for microbial growth are substantially changed, such as the case with introduction of groundwater contaminants into the aquifer, microorganisms must adapt themselves to the new conditions. When the contaminant offers a potential substrate to support the microorganisms, the microorganisms must adjust, depending on how different the contaminant is from the normal substrate. Microbial populations can adapt to new substrates by induction of specific enzymes not present before exposure. The composition of the population can also change to reflect an increase in the number of organisms able to metabolize newly available substrates (Chapelle, 2001). Through natural selection, assuming that the contaminant offers a significant substrate, microorganisms that can utilize the contaminant in the most efficient way will thrive. The composition of the microbial population will then change as it becomes more acclimated to the presence of the contaminant. Therefore, rates of biodegradation via reductive dechlorination will be a partial function of the acclimation of indigenous microorganisms to the contaminated conditions. In turn, this implies that biodegradation rates will change over time as acclimation proceeds, with the potential for increasing substantially as microorganisms become more acclimated (Chapelle, 2001).

Figure 6 represents the cross sectional area of the WPAB constructed wetland and sections where piezometers were introduced for measurement of PCE concentration.

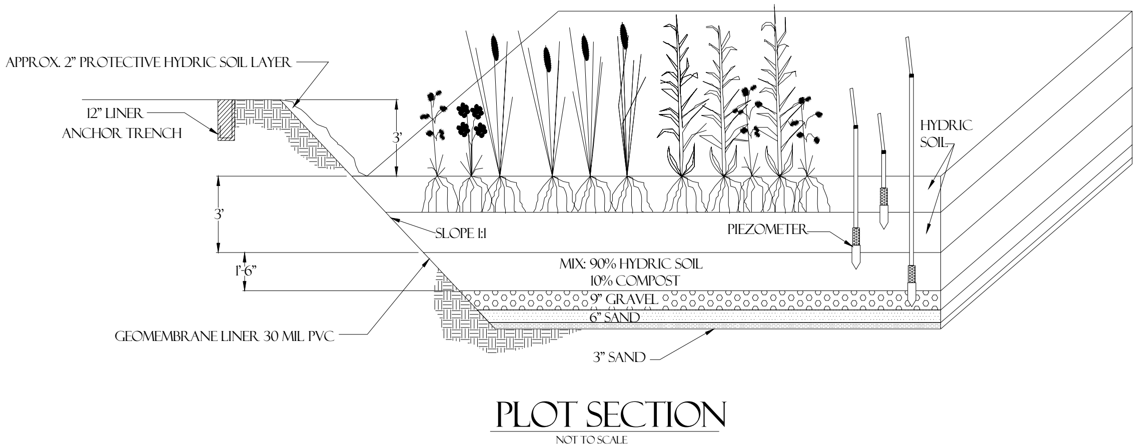


Figure 6. WPAFB Constructed Wetland Cross Section (Bugg, 2002)

Many *in-situ* remedies rely on the mechanisms of biodegradation to treat chlorinated solvents. Both aerobic and anaerobic microorganisms are capable of using contaminants as sources of carbon and energy for growth, thus are the driving force behind the remediation of chlorinated solvents. DNA analysis provides a methodology for bacterial identification.

DNA Analysis

Recent research suggests that only 1% of the microorganisms present in soil are readily culturable (Edwards, 1999). However, improvement in molecular techniques (DNA analysis) has aided in the identification of microbial communities in soil samples. Among these techniques are 16S rDNA sequences, Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) (Taylor, 1997). The 16S rDNA gene has been used extensively since standard phenotypic methods of identification have failed to identify bacteria (Woese et al., 1990). The advantages of using 16S rDNA are primarily due to its universal distribution among bacteria and the many species-specific variable regions that affords the establishment of public-domain databases for bacterial identification (Weisburg et al., 1991). In PCR a target sequence of a gene or segment of DNA is amplified exponentially. During RFLP restriction enzymes digest the DNA

at a sequence-specific site. If a specific restriction site occurs in more than one location on a DNA molecule, a restriction enzyme will make a cut at each of those sites, resulting in multiple fragments. Therefore, if a given piece of DNA is cut with a restriction enzyme whose specific recognition code is found at two different locations on the DNA molecule, the result will be three fragments of different lengths. The length of each fragment will depend upon the location of the restriction sites on the DNA molecule. DNA that has been cut with restriction enzymes can be separated and observed using agarose gel electrophoresis. PCR and RFLP allow for analytical interpretation of a bacteria community without subjecting the organism to selective biases such as optimal temperature or unfamiliar substrate imposed by culture-based identification methods. These two techniques afford a researcher the ability to better analyze a bacterial community at a molecular level and in its natural habitat. This analysis can provide detailed information of the structure of the bacterial community and how the organisms interact in processes such as natural attenuation of polluted sites. The process of extracting bacterial DNA from soil samples is extensive and requires several steps. The following section illustrates how DNA is extracted and identified from soil.

DNA Extraction

Many extraction techniques have been developed to provide quality microbial DNA from soil. These commercial products and techniques reduce the amount of impurities in DNA extracted from soil. These impurities can inhibit further molecular procedures such as PCR. Impurities such as humic acid diminish the quantity and quality of binding sites of nucleotides thus preventing replication of DNA; the basis of PCR. For this research, a soil extraction kit (MoBio, Inc., Carlsbad, CA) was used to isolate DNA from soil.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a method that allows exponential amplification of short DNA sequences within a longer double stranded DNA molecule. PCR entails the use of a pair of primers that are complementary to a defined sequence on each of the two strands of the DNA. These primers are extended by a DNA polymerase so that a copy is made of the designated sequence. After making this copy, the same primers can be used again, not only to make another copy of the input DNA strand but also of the short copy made in the first round of synthesis. This leads to exponential amplification. In essence, each cycle denatures, anneals, and extends targeted DNA segments, resulting in an exponential increase in DNA (Edwards, 1999). In this research the above procedures were followed with the exception of an additional initial denaturation of 95° C for 15 minutes to activate the polymerase (HotStart Taq polymerase, Qiagen, Inc., Valencia, California). After the replication cycles are completed, the PCR product is analyzed with agarose gel electrophoresis with ethidium bromide stain for visualization. The entire cycling process takes approximately 3.5 hours to complete.

Primer Design

The 16S rRNA consists of 1542 bp and is found within the genomes of all bacterial species. The gene has conserved regions as well as variable regions. Differences in the latter can give insight to bacterial groups within wetlands allowing for fingerprinting of the community. The uniqueness of this gene lies on its utility to identify bacteria in their natural environment, therefore bypassing the need to use traditional culture base-methods. Over 10% of bases in the 16S rDNA gene are totally conserved (within a sample of 500 bacterial sequences; Baker, 2003). The majority of these conserved bases are, however, not adjacent to each other and thus form no continuous conserved regions for universal priming. The longest string of totally conserved bases is between

positions 788 and 798, but in most areas of the gene absolutely conserved bases are found in strings of less than 4. Thus, no primer of sufficient length can be designed that is a 100% match to all bacterial 16S rRNA gene sequences (Baker, 2003). For this research, the forward primer (f)E8F (10 bp) was annealed at the 5' end of the gene, while the (f)AU1406R (17 bp) was annealed 3' end in order to amplify the entire length of the targeted DNA (1406 bp). Primer (f)AU1406R was used due to its ability to give good results for soil samples taken under in-situ conditions (Baker, 2003).

Electrophoresis

Gel electrophoresis separates a mixture of molecules through a stationary agarose gel in an electrical field (Edwards, 1999). Since DNA is negatively charged an electrical current forces the DNA to migrate through the gel. Based on their weight, larger molecules travel slower and remain closer to the top of the gel while smaller molecules move to the bottom thus creating unique separation that was visualized and compared to known markers (ladder). Figure 7 depicts a standard electrophoresis gel.

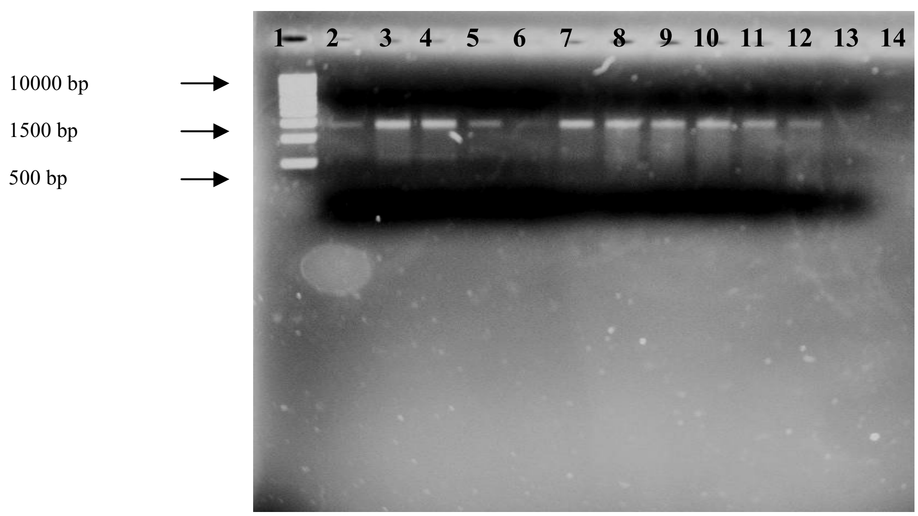


Figure 7. Agarose Gel Electrophoresis of DNA with known ladder

RFLP

Restriction fragment length analyses use restriction enzymes (RE) to cut DNA at specific 4-6 bp recognition sites (Clement, 1998). Sample DNA is cut or "digested" with one or more REs and resulting fragments are separated according to molecular size using gel electrophoresis (Aviniss, et. al, 1994). Molecular size standards are used to estimate fragment size. Ethidium bromide staining is used to reveal the fragments under UV (260 nm) light. Differences result from base substitutions, additions, deletions or sequence rearrangements within RE recognition sequences (Edwards, 1999). Restriction fragment length polymorphism (RFLP) is most suited to studies at the intraspecific level. Presence and absence of fragments resulting from changes in recognition sites are used identifying for species or populations. The restriction process takes between 2 and 4 hours. Fig 8 shows the basic steps in RFLP.

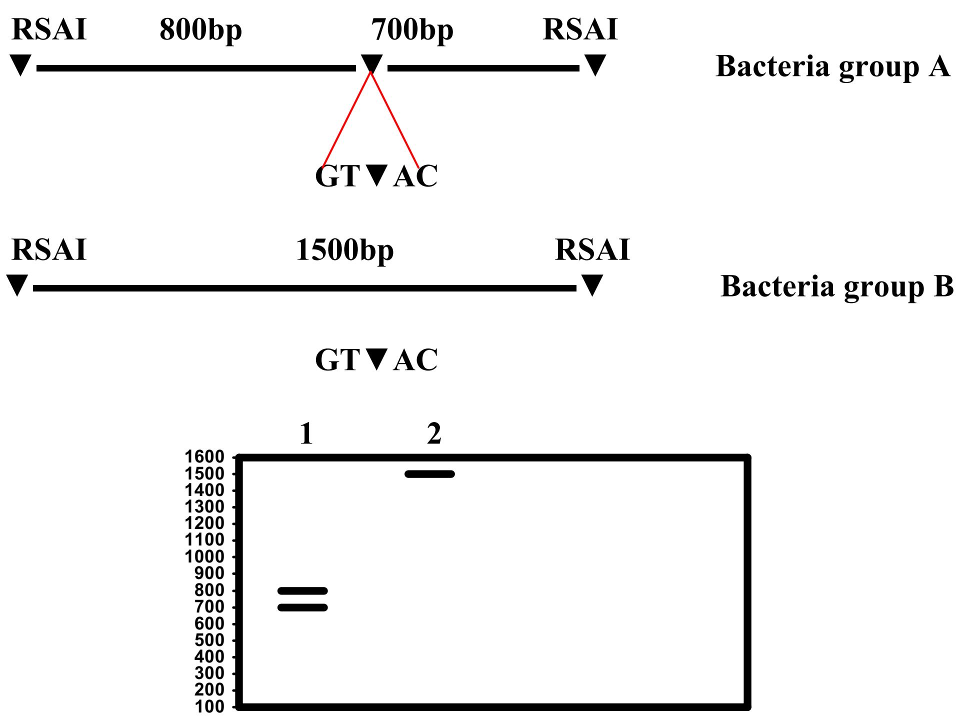


Fig 8. Steps in RFLP. In bacteria group A, Restriction enzyme RSAI cuts the DNA at recognition site GT▼AC making to distinct profiles (lane 1). In bacteria group B, the DNA sequence does not have the recognition site thus the sequence is not digested (lane 2).

Summary

Complete reductive dechlorination of PCE has been observed more commonly in mixed cultures or in field studies rather than pure cultures (Flynn et al., 2000). Knowledge of the bacterial groups' profiles in these environments would allow for future understanding of the mechanism that these organism are subjected to and the proper mix of organisms to incorporate when constructing a wetland for the purposes of treating chlorinated ethenes. Research suggests that at least two populations of bacteria are responsible for sequential dechlorination of tetrachloroethylene (PCE) to ethane (Lorah, 1999). Knowledge of the biodegradation pathways of PCE and associated bacterial communities are of great importance. The communities can be studied through genetic techniques i.e., PCR/RFLP since these methods provide a means to observe bacterial communities in their natural habitat.

III. Methodology

Experimental Overview

Previous research focused on identifying procedures inherent to reducing chlorinated compounds into less dangerous forms through the use of wetlands. This research however focuses on identifying the bacterial community associated in wetlands through PCR and RFLP analysis with the hope of identifying restriction patterns associated with bacteria that naturally dechlorinate these compounds.

Soil Sampling

3" diameter, 45 inches long soil cores were taken from both the treatment and a naturally-occurring wetland during the summer, fall, winter, and spring using a precut and labeled aluminum cylinder. The cylinders were driven into the soil with a sledge hammer through all three depths of the wetlands and retrieved with a hand-held winch mounted on a tripod. After retrieval from each wetland, samples were covered with ziplock bags and transported to Wright State University greenhouse within an hour. Cores were removed from the aluminum cylinders by means of a table saw. Cores were split in half and then quarters and 10g samples were aseptically removed from the middle and the entire length of each core, to prevent the contaminated, external regions of core from touching the samples to be analyzed. A total of twelve 10g samples from each of three depths (0 to 9, 9 to 27, and 27 to 45 inches) and each season were taken. Each sample was manually homogenized by 100 strokes with a sterile rod. 0.5g was taken from the 10g samples in quadruplicates, and two 0.25g samples were removed from two of the 0.5g aliquots to be processed as describe below. All remaining soil was archived at -20°C for future use.

Laboratory Methods

Successful PCR requires adherence to strict protocols in order maintain uncontaminated samples and minimize DNA degradation. Therefore during the process of soil retrieval, transport, and handling, sterile and optimum temperature conditions were maintained. Soil samples were taken from WSU laboratories to AFIT and placed at 4° C within 1 hour. Sterile conditions during sample processing were maintained by using a laminar flow hood equipped with UV capabilities and cleaned daily with 10 % bleach and 70 % ethanol. All equipment, glassware, and plasticware were autoclaved. Latex gloves were frequently changed and cleaned with bleach and ethanol solutions to prevent cross-contamination of samples during processing.

DNA Extraction from Soil

The preferred method of DNA extraction and purification for two silt loam soils and silt loam wetlands sediment was the beadbeat (Power soil by MoBio Corp. Carlsbad, California). These kits contained the optimal extraction, cloning and purification techniques that were statistically found superior to other methods (Miller et al, 1999). Before extracting the DNA from the soil, the laminar flow hood was sterilized with 10% bleach and 70% ethanol solution and exposed to UV light for 15 minutes. Latex gloves were also used and exchanged in between samples and also sterilized with bleach and ethanol. All fifty 0.25g samples were placed into a 2ml power bead tube and vortexed for 1 min. 60µl of cell membrane rupturing solution containing SDS was added and vortex for 1 min in order to release the DNA. The samples were centrifuged at 10,000g for 1 min and the supernatant transferred to a sterile collection tube. 250µl of a cleaning solution was added to remove humic material and then centrifuge for 1 min at 10,000g. The supernatant was collected and 200µl of a second cleaning solution was added, vortexed and incubated at 4°C for 5 minutes. Samples were centrifuged for 1 min. at 10,000g and the supernatant collected. 1.2ml of salt

solution was added to the supernatant and vortex for 5 sec in order to bind DNA to silica and release other impurities. Approximately 675µl of supernatant was added to a 2ml spin filter and centrifuge for 1 min. at 10,000g discarding the liquid after cycle completion. This step is performed until all supernatant is used; this allows the DNA to bind to the filter membrane and other impurities to pass through. 500µl of ethanol based solution was added to the spin filter and centrifuge for 30 sec. at 10,000g in order to further clean the DNA. The liquid was discarded and the spin filter is centrifuge for 1 min. at 10,000g. The spin filter with DNA was transferred to a sterile 2ml tube and the DNA was released from the silica with 100µl of eluting solution. Samples were centrifuged for 30 sec. The spin filter was discarded and the DNA was stored at -80°C. See appendix A for detail protocol procedures.

PCR

The following modification to literature review procedures on PCR amplification procedure was used. Amplification was performed using domain specific 16S rDNA primers (f)E8F (5'AGAGTTTGATCCTGGCTCAG3', *E. coli* 16s rRNA positions 8 to 27) and (f)AU1406R (5'GACGGGCGGTGTGTGTRCA3', *E. coli* 16s rRNA positions 1390 to 1408). Ideally, DNA should have been quantified spectrophotometrically, but in this research, the concentration was empirically determined. Measuring the DNA concentrations in the template DNA extracts months later, it was determined that their concentrations ranged from 5.55ng/µl to 216ng/µl, and the average used for PCR samples was 51.17ng/µl. The following is an example of the calculations performed to obtain the DNA mass for PCR analysis.

10.44 ng/µl X 3ul used in PCR = 327ng of template DNA.

0.5 µM of forward primer (f)E8F, 0.5µM of reverse primer (f)AU1406R, 62.5 units of *Taq* polymerase, and enough D.I. was added to each sample to reach a volume of 25µl of reaction. HotStart Taq polymerase (Qiagen, Inc., Valencia, California) was used specifically due to its ability to yield more DNA from soil samples (Qiagen Inc., Valencia, California). During PCR cycling, an additional denaturation step of 95°C was needed to activate this polymerase. Samples were process in a MWG Primus 25 Thermal cycler (Genomic Technology, Ebegsgerg, Germany) with the following program establish based on the product size and polymerase used.

- Lid Temp = 105°C
- Initial denaturation: T = 95°C for 15 minutes. Due to the unique make attachment of an antibody preventing false denaturation, an extended denaturation time was necessary.
- Denaturation: T = 94°C for 1 minute.
- Annealing: T = 46°C (Empirically derived) for 1 minute.
- Extension: T = 72°C for 1:30 minutes.
- 29 repetitions for a total of 30 cycles.
- Incubation: T = 4°C.

The annealing temperatures were estimated based on the size and composition of the primers as follows:

<u>(f)E8F</u>

$$T_m = 4(G+C) + 2(A+T)°C = 4(6+4) + 2(4+6)°C = 60°C$$

<u>(f)AU1406R</u>

$$T_m = 4(G+C) + 2(A+T)°C = 4(6+7) + 2(2+4)°C = 64°C$$

Empirical optimization then was done to arrive at 46°C (Baker). See HotStart Taq Master Mix protocol in Appendix B for further details. Approximately 3µl of PCR product, 4µl of 10X buffer, and enough D.I. to reach a volume of 25µl reaction was added to each well in the gel box containing a 1% agarose and ethidium bromide. The samples were run 1 hour at 110 volts. Bands were visualized by using Kodak 1 D Analysis software (Gel Logic 200 Imaging system, Kodak, Inc, CA) and compared to a known 100bp and occasionally 1kb and 10kb ladders (Promega, Corporation, Madison, WI).

PCR Purification

PCR products are commonly purified to remove excess nucleotides and primers. Without this process, the concentration of DNA in samples can be inaccurately represented thus skewing follow-on analysis. A membrane-based system that binds up to 50µg DNA was used and it allows for recovery of isolated DNA fragments of PCR products in 20 minutes. A Promega clean up kit (Promega, Madison, WI) was used and followed accordingly.

Mix a membrane wash solution by adding 375ml of 95% ethanol to 1000ml membrane binding solution. Add an equal volume of membrane binding solution to each volume of PCR sample into a minicolumn. Place a minicolumn into a 1.5 ml collection tube with PCR product and incubate at room temperature for 1 min. Centrifuge sample for 1 min at 16,000g. Remove minicolumn and discard liquid. Wash column by adding 70µl of membrane washing solution. Centrifuge for 1 min at 16,000g. Empty collection tube and place minicolumn back into tube and repeat procedures with 500µl of membrane wash solution and centrifuge for 5 min. Repeat again with remaining wash solution (should be 3 washes). Remove minicolumn, empty liquid, and centrifuge for 1min at 16,000g. Transfer minicolumn to a sterile 1.5ml tube and apply 50µl of D.I. Incubate at room temperature for 1 min. Centrifuge each sample for 1 min and discard minicolumn. Store eluded DNA at 4°C. The purified DNA was then used for digestion without further manipulation. See appendix D for detail information on DNA purification.

DNA Concentrations

Unsuccessful gel electrophoresis results were common place due to the mechanical homogenizing of soil samples thus potentially unequally distributing DNA in samples unequally, the amount of MasterMix (Taq Polymerase) used, high DNA concentrations in template, or the

presence of inhibitory materials. Empirically it was determined that approximately 50.17ng/µl of template DNA was sufficient for a positive result during PCR analysis and subsequent gel electrophoresis. For RFLP "equal loading" of digested DNA samples was necessary to accurately represent bacterial community concentration in each wetland, season, and depth. Spectrophotometer (NanoDrop Technologies, Wilmington, DE) readings showed that each sample of cleaned DNA from PCR varied significantly. A comparison of protein to DNA was conducted since each absorbs UV light at different wavelengths. DNA's peak absorbance is at 260 nm and protein's is at 280 nm. The purity of the DNA solution can be determining through the ratio of DNA to protein, i.e., the $A_{260}/A_{280.}$ An A_{260}/A_{280} ratio of 1.7-2.0 is recommended and a ratio of greater than 1.9 indicates relatively pure DNA concentration (Graham, 2001). Table 7 illustrates DNA concentration for each cleaned PCR sample.

<u>RFLP protocol</u>

Restriction Fragment Length Polymorphism (RFLP) analysis is a method of comparative community analysis. In this research the 16S rDNA of bacterial communities in wetlands was digested with *Rsa*I and fingerprinted. The DNA concentration was empirically derived from the ratio of DNA to protein concentration (ng/µl) multiplied by the volume (µl) of template DNA. The range of concentration was between 496ng/µl to 22.7ng/µl and the average used for reaction was 77.9ng/µl. The following is an example of the calculations performed to obtain the DNA mass for RFLP analysis.

77.9 ng/µl X 15ul in the *Rsa*I digest = 1168.5ng of PCR product.

1168.5ng of PCR product was placed in a sterile Eppendorf tube with 2µl of 10X buffer (Promega, Madison, WI), 25µg/µl of Bovine Serum Albumin Acetylated (BSA); Promega, Madison, WI), and 0.5units of *Rsa*I (Promega Madison, WI)). The volume of D.I. varied within each samples but

enough D.I. was added to reach a reaction volume of 25.5µl. Samples were digested in a dry incubator (Quincy Lab Inc, IL) at 37°C for 2.5 hours and ran on a 2.5% agarose gel for 3.5 hours at 70 volts. Samples were visualized through ethidium bromide and UV by using Kodak 1D Analysis software (Gel Logic 200 Imaging system. Kodak, Inc, CA). A 100 bp ladder was used to compare all digested sample sizes. For this research, the ratio of DNA to protein was used as the target concentration for loading into RFLP reactions instead of the mass of each sample causing unequal loading. Samples with uneven intensity when viewed on the gel can lead to skewed results and data misinterpretation.

Summary

The results of the procedures described in Chapter 3 are provided in order to give detail characterization of microbial distribution in the top 45 inches of the wetlands. Polymerase Chain Reaction and Restriction Fragment Length Polymorphism were performed in accordance with documented protocols and slightly adjusted based on the experimental procedure needed to accurately represent inherent conditions associated to wetlands thus insuring maximum analytical output. Ultimately these techniques can be used to better understand the microbial community composition and as a precursor to remediating polluted sites by means of wetlands.

IV. Results

Polymerase Chain Reaction (PCR)

Unsuccessful results during PCR were common. Some of the samples fail to travel the total distance of the gel box when compared to the pre-established DNA marker, “ghosting” made recognition of bands difficult, and lack of band visibility when photographed were among the negative results seen. Possible causes included a high DNA concentration from template DNA, pipetting errors during extraction, high concentration of humic material or possible contamination of the reagents. In order to find the right concentration of DNA empirically tests were conducted thus concluding that 50.17ng of template DNA yielded the best results on the gel. Appendix H displays the concentration of reagents used during PCR for all cores in wetland samples. Figures 20a through 20v depicts all samples ran on the gel.

Hypotheses/corrective actions

Between 1.75 and 2.00 on the A_{260}/A_{280} protein/DNA ratio was used as guidance for positive PCR results and an average of 50.17ng of template DNA provided generally good results. However, the clarity of DNA bands during gel electrophoresis varied. This anomaly was seen primarily in the top 12 inches of the wetlands which is consistent with higher concentration of humic material. In order to remediate this discrepancy, strict adherence to the soil extraction kit needs to be followed.

Restriction Fragment Length Polymorphism (RFLP)

Lack of restricted bands (absence of DNA) during the initial digestion was common. Possible causes included a low DNA concentration from PCR, pipetting errors during extraction, mishandling during DNA purification and low percentage of agarose gel during electrophoresis. In order to maximize results, the average concentration of DNA of all samples was added to each reaction by first calculating the ratio of DNA to protein concentration; this was accomplished by running all samples under the spectrophotometer. Between1.75 to 2.00 on the A_{260}/A_{280} protein/DNA ratio was used as guidance for positive RFLP results or ~1168.5ng of clean PCR. An empirical test was conducted concluding that a 2.5 % agarose gel for 3.5 hours at 70 volts yielded the best results. Appendix I display the concentration of reagents used during restriction. Figures 21a through 21v depict the results during restriction.

Hypotheses/corrective actions

Although 1168.5ng of cleaned PCR provided good results, the clarity of DNA bands during restriction varied. This anomaly was seen across all depths which is consistent with unequal distribution of bacterial community due to manual homogenization of samples. In order to remediate this discrepancy, strict adherence to the Promega wizard clean-up kit was followed. Additionally, the DNA mass of each sample should be used instead of the A_{260}/A_{280} protein/DNA ratio in order to have a true "equal loading".

Population Comparison

When comparing the treated and naturally occurring wetland, the bands of the following sizes, were observed in general: 1400, 1300, 1000, 900, 850, 800, 550, 500, 450, 400, 300, 250, 200, and 150bp between wetlands collectively. However, the band differences between each site was 1400,

100, 850, 550, and 300bp. When analyzing depth, only 550 and 300bp were seen as differences between all three depths in both wetlands analyzed. For seasonal comparison band sizes differences were seen at 1400, 1000, 850, 550, and 300bp. Although significant differences where not seen through wetlands, seasons or depths, certain group base pairs (bp) did exhibit changes in pattern, and intensity and must be further evaluated; specifically, Wright Patterson wetland, Winter sample, core 2 section 2, Valle Greene, Spring sample, core 5 section 2, Valle Green, Winter sample, core 7 section 2, and Wright Patterson, Fall sample, core 8 section 3. Table 8 and 9 respectively illustrates bp differences and comparison between wetlands, depth, and seasons. When comparing Valle Greene to WPAFB band seasonal diversity the sum of all base pairs (annual) between wetlands was divided by the total base pair of the individual season; for example,

8800bp overall seasonal/8500bp overall fall samples X 100 = 96.6% diversity (fall)

The above calculation was conducted for depth comparison except for the total of both wetland depths was divided by the total of a section depth i.e., 0 to 9 inches in the following manner:

8600bp overall depth/6350bp overall-0 to 9 inches X 100 = 79.37% diversity (0 to 9 inches)

Similar calculations were conducted for overall wetland diversity between Valle Greene and WPAFB. The computation was performed as follow: Sum of both wetland base pair (season and depth) divided by total-individual wetland base pair

9050bp overall wetlands/ 9050bp overall Valle Greene X 100 = 100% diversity (Valle Greene)

For ease of comparison, the following graphical representations of all wetland RFLP bands are provided; tables 8 and 9 provide actual images of RFLP band patterns.

Comparison of Valle Greene to WPAFB at all seasons and depth

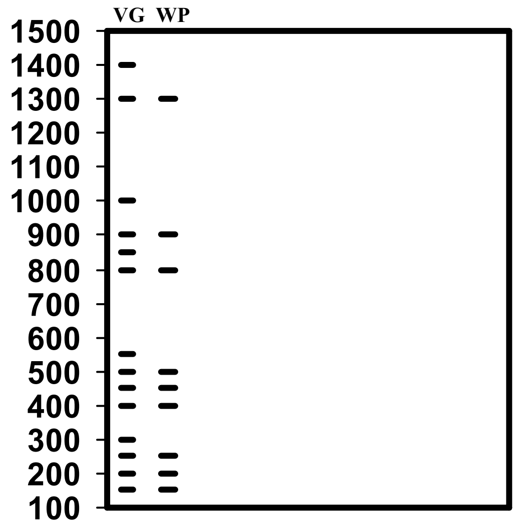


Fig 9. Y-axis represents relative base pair size. Lane 1=Valle Greene VG, lane 2=Wright Patterson (WP)

Comparison between seasons at all depth and wetlands

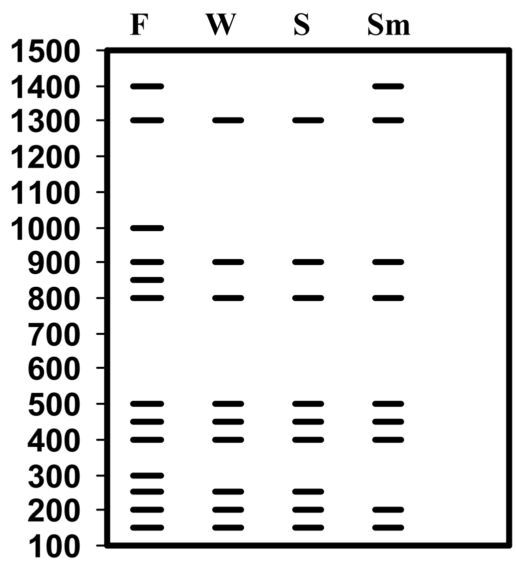


Fig10. Y-axis represents relative base pair size. Lane 1=fall (F), lane 2=winter (W), lane 3=spring (S), lane 4=summer (Sm).

Comparison between depths at all seasons and both wetlands

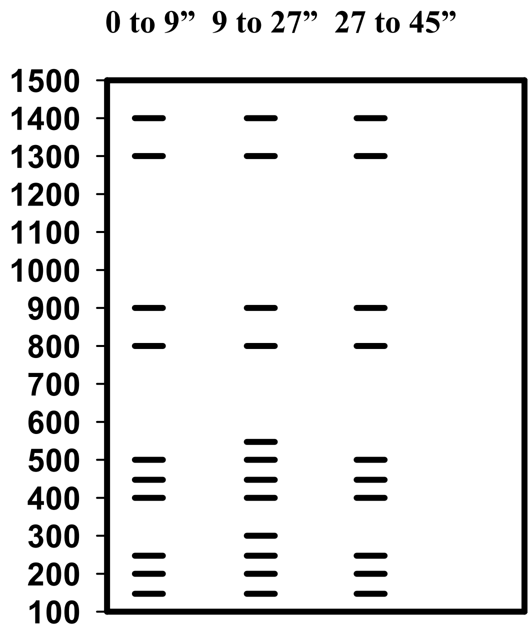


Fig 11. Y-axis represents relative base pair size. Lane 1=0 to 9 inches, lane 2=9 to 27 inches, lane 3=27 to 45 inches

Comparison between 0 to 9 inches in depth for all wetlands and seasons

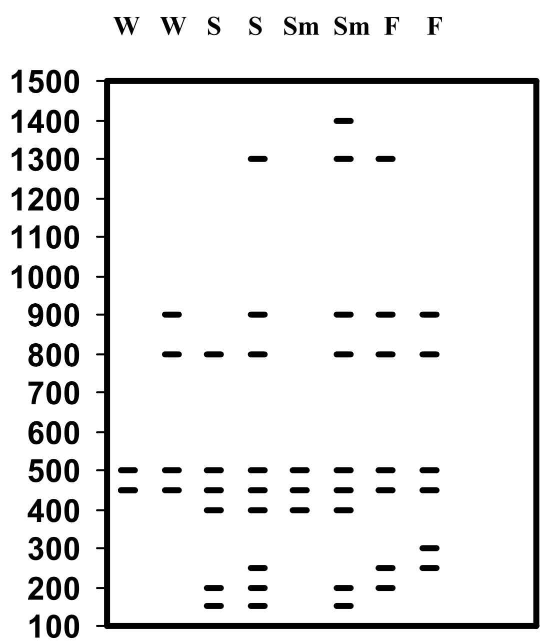


Fig 12. Y-axis represents relative base pair size. Lane 1=V.G. (W), lane 2=W.P. (W), lane 3=W.P. (S), lane 4=V.G. (S), lane 5=W.P(Sm), lane 6=V.G. (Sm), lane 7=W.P. (F), lane 8=V.G. (F). Note V.G. =Valle Green and W.P=Wright Patterson. W=winter, S=spring, Sm=summer, F=fall

Comparison between 9 to 27 inches in depth for all wetlands and seasons

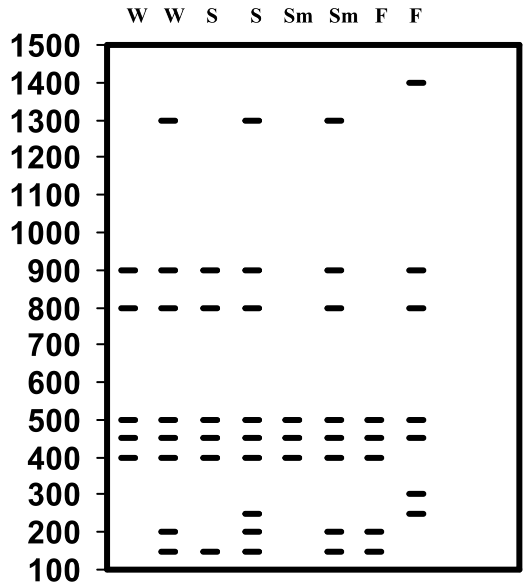


Fig 13. Y-axis represents relative base pair size. Lane 1=V.G. (W), lane 2=W.P. (W), lane 3=W.P. (S), lane 4=V.G. (S), lane 5=W.P (Sm), lane 6=V.G. (Sm), lane 7=W.P. (F), lane 8=V.G. (F). Note V.G. =Valle Green and W.P=Wright Patterson. W=winter, S=spring, Sm=summer, F=fall

Comparison between 27 to 45 inches in depth of all wetlands and seasons

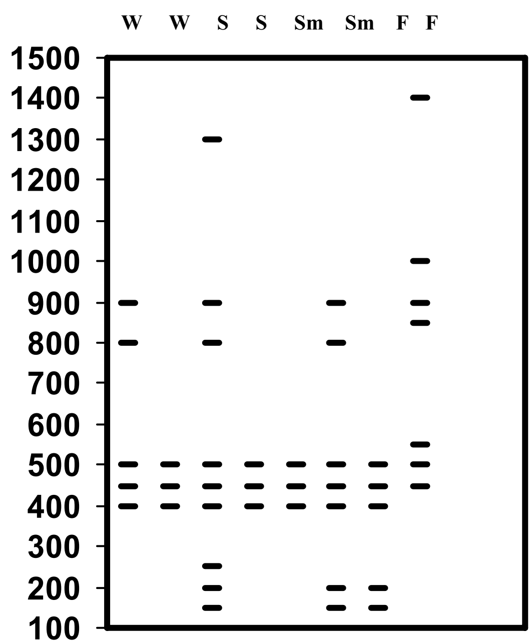
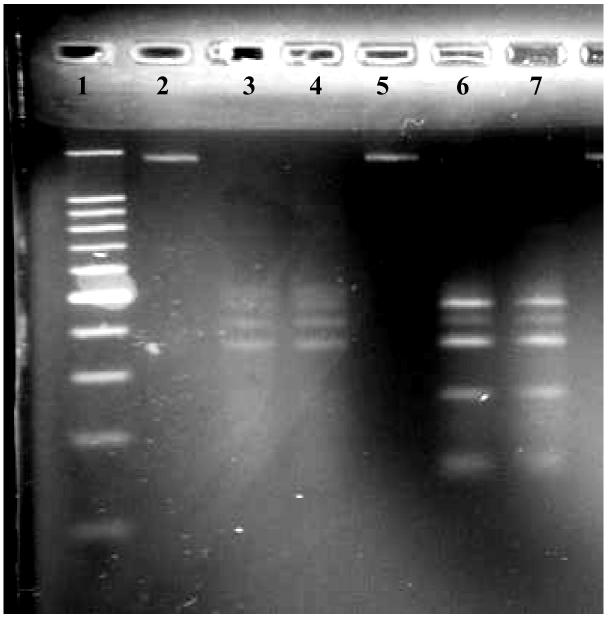


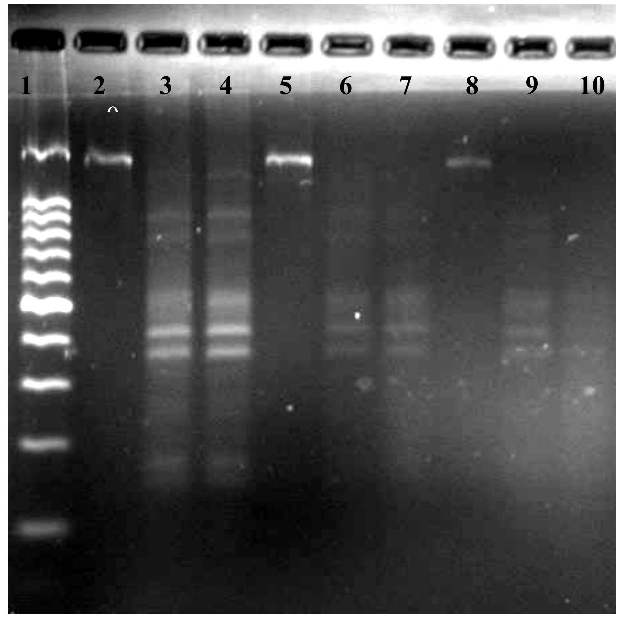
Fig 14. Y-axis represents relative base pair size. Lane 1=V.G. (W), lane 2=W.P. (W), lane 3=W.P. (S), lane 4=V.G. (S), lane 5=W.P(Sm), lane 6=V.G. (Sm), lane 7=W.P. (F), lane 8=V.G. (F). Note V.G. =Valle Green and W.P=Wright Patterson. W=winter, S=spring, Sm=summer, F=fall

Sample intensity comparison

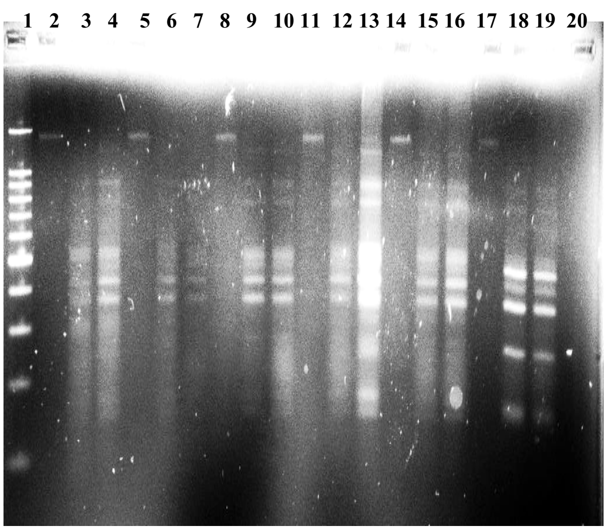
a.



b.



c.



d.

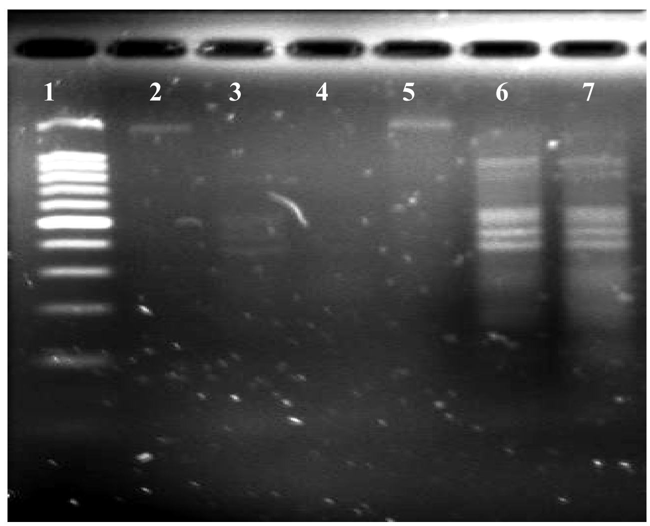


Fig15. Image a, b, c, and d were taken at different times. Lanes 1 on all images correspond to 100bp ladder. Figure a, lanes 6 and 7 correspond to Wright Patterson AFB wetland, winter sample core 2, section 2. Figure b, lanes 6, 7, 9, and 10 correspond to Valle Greene wetland summer sample core 2, section 2. Figure c, lanes 18 and 19 correspond to Wright Patterson AFB wetland fall sample core 8, section 3. Figure d, lanes 6 and 7 correspond to Valle Greene wetland, spring sample core 5, section 2.

In this research relative band diversity was estimated for both wetlands, between each depth, and throughout the seasons. The calculation were performed as follows: 1. Wetland band diversity was calculated as total base pair size of both sites divided by total individual site giving the following: Valle Greene 100% band diversity and WPAFB 54.7%, 2. Seasonal band diversity, was calculated by using the sum of seasonal bands of all wetlands divided by the total seasonal band size of each season giving the following: fall 96.6%, winter 62.5%, spring 56.25%, and summer 69.32%, 3. Depth band diversity was calculated as total base pair depth of both wetlands divided by total individual wetland depth giving the following: 79.37% in the first depth (0 to 9 inches), 100% in the second depth (9 to 27), and 79.37% in the last depth (27 to 45 inches). Figure 16 depicts the tables representing relative diversity used in this research.

Relative band diversity seasonal comparison

Base pair total		**fall**	**winter**	**spring**	**summer**
8800		96.6%	62.5%	56.25%	69.32%
Fall total	**8500**				
winter	**5500**				
spring	**4950**				
summer	**6100**				

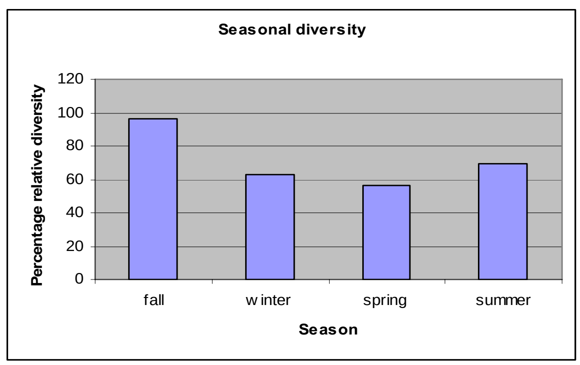


Fig16a. Relative band diversity across seasons. Calculated as total annual diversity divided by total season diversity

Relative band depth diversity comparison

Base pair total		section	section	section
8600		**0 to 9**	**10 to 27**	**28 to 45**
0 to 9	**6350**	79.37%	100%	79.37%
9 to 27	**8600**			
27 to 45	**6350**			

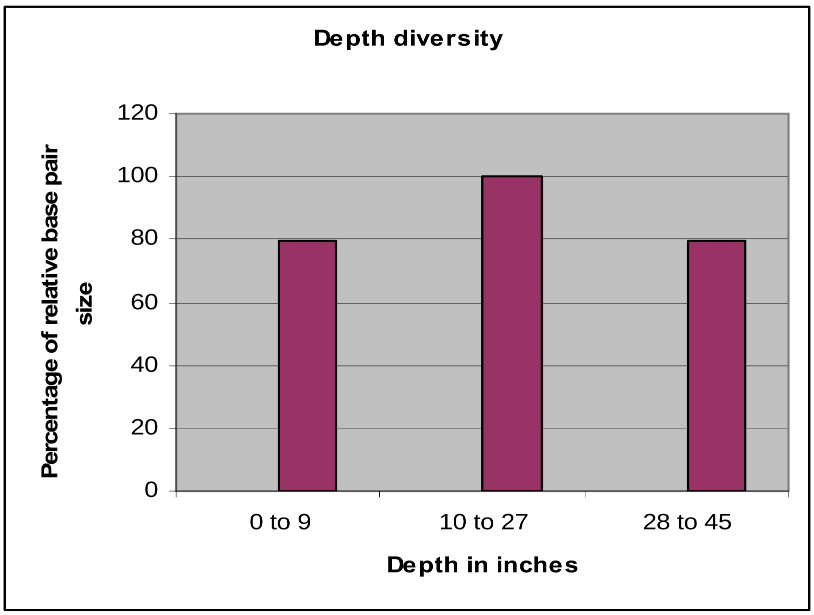


Fig16b. Relative band diversity across depth. Calculated as total depth diversity divided by total section diversity

Relative band wetland diversity comparison

Base pair total	Valle Greene	WPAFB
9050	100	54.7
Valle Green 9050		
WPAFB 4950		

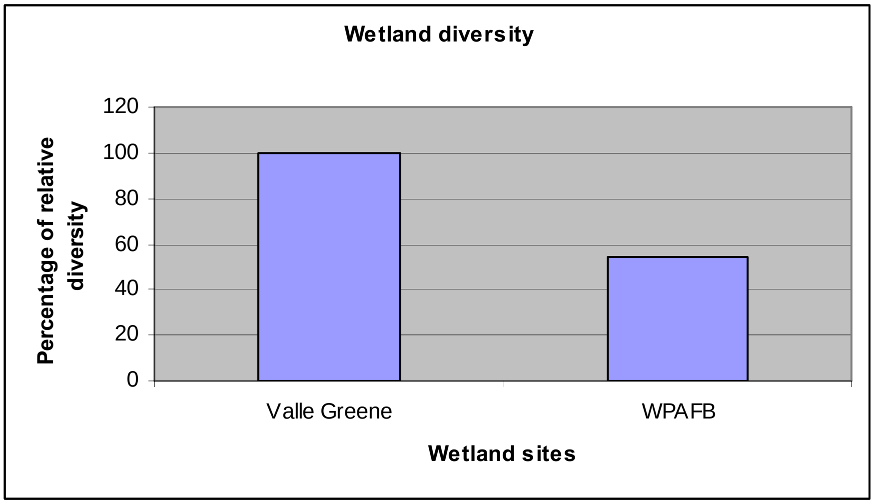


Fig16c. Relative band diversity between wetlands; calculated as total annual band diversity divided by total season diversity.

Conclusion and recommendations for further study

Effort Strengths

This research provided a much better characterization of the overall spatial differences, or lack thereof, of the microbial community within WPAFB's and the Beavercreek wetlands than culture-base methods. It served as a foundation for a better assessment and analytical interpretation of the bacterial community especially since conventional methods i.e., culturing, rely on optimal conditions for bacterial growth thus interjecting biases into the overall process. By performing PCR/RFLP natural bacterial processes are not ignored, actual characterization of the bacterial community is highlighted, and potential optimum environmental conditions i.e., substrate, requirements can be identified leading to accurate data.

Effort Limitations

This effort proved weak in providing complete bacterial groups accuracy since PCR introduces unique biases and errors due to extraction methods involving soil sampling positions. Further, uneven distribution of DNA content due to cell lysis via Beadbeat method, potential inhibitors such as humic acid and heavy metals reducing overall DNA content, DNA deterioration during freezing and thawing, primer design, and potential sample contamination all contributed to potential misinterpretation of findings. Visualization of bands and their intensities during RFLP also present limits to full data analysis as this method is subjective and lend itself to multiple interpretations. Additionally, band ghosting and unidentifiable restriction patterns on gels specifically at less than 100bp bands were common thus forcing to disregard this data as negligible but unfortunately not fully representing the bacterial profile in either wetland.

Recommendation for further study

Field sample retrieval and initial laboratory efforts was challenging; specifically establishing proper protocols for depicting DNA concentration. In order to maximize time, DNA samples should be immediately analyzed by comparing the DNA to protein concentration on the spectrophotometer. Although positive results were seen, there is a need to apply more than one technique to confirm the results whenever techniques like PCR are used. For instance, confirmation of base pairs sizes with different restriction enzymes i.e., *HhaI* or *Msp*I is a must Brunk et al (1996). This guarantees that biases in restriction sites are minimized thus validating the process even further. Additionally, by using different restriction enzyme a sensitivity comparison can be made to evaluate which enzyme is more effective.

Future work evaluating the sensitivity of Terminal Restriction Fragment (TRF) through fluorescent primers to better depict the actual base pair sizes is also a must. By employing this technique precise fingerprinting of bacterial groups can be summarized and then use for follow-on research such as cloning and sequencing.

Conclusion

PCR/RFLP based methods were found to be an effective tool for rapidly fingerprinting bacterial communities in wetlands because of their ability to identify differences in communities without the need for sequencing and cloning amplicons. Visual determination of the sizes of the restriction fragments relative to the position of the molecular size standards was reliable. After several gels, identifying similar bp sizes between 900, 800, 500, 450, and 400bp became consistent and predictable. On the other hand, fragments smaller than 100bp were not as easily identified. For the purpose of this research, bands below 100bp were considered negligible and not incorporated in the results. For ease of interpretation in-between bands i.e., 170, 290 were rounded to the lowest and highest number respectively and incorporated in the results. This research

provided the following number of base pairs bands between both wetlands 1400, 1300, 900, 850, 800, 700, 550, 500, 450, 400, 300, 250, 200, and 150bp. However, the differences between each site was 1400, 1000, 850, 550, and 300bp. When analyzing depth; only 550 and 300bp were seen as differences between all three depths in both wetlands. Differences of 1400, 1000, 850 and 300bp were also seen between seasons in both wetlands and all depths. The intensity of some bands relative to others also differed, and must be further analyzed. Specifically, Wright Patterson's treated wetland; winter core section 2 and fall's core section 3 as well as Valle Greene's winter core section 2 and springs core section 2 displayed a different bacterial community profile thus implying a need for further evaluation of the bacterial composition. These differences in intensity among the fragments seen in the winter and fall cores (WPAFB) can further indicate bacteria which can be correlated with the availability of substrate in the treated-PCE contaminated site. Valle Greene's spring and summer cores also suggest a difference in bacterial growth that should be evaluated through other means i.e., root zone comparison. For overall seasonal band diversity, the annual base pair total of both wetlands was compared by dividing it by each total seasonal band size giving the following: fall 96.6%, winter 62.5%, spring 56.25%, and summer 69.32% suggesting that optimal conditions for relative band diversity is seen in fall perhaps due to moderate temperatures or even microbial community reaching balance within each other. As temperature decreases (winter), bacterial more resistant to colder temperatures thrive and population diversity decreases reaching to its lowest diversity level in the spring. Band diversity comparison through all wetland depths was calculated as total base pair depth of both wetlands divided by total individual wetland depth giving the following: 79.37% in the first depth (0 to 9 inches), 100% in the second depth (9 to 27 inches), and 79.37% (27 to 45 inches) in the last depth, suggesting perhaps that more substrate is present between 10 and 28

inches of all wetlands; this availability of substrate can be further evaluated in follow-on GCE testing. Band diversity comparison between wetlands was calculated as the total base pair size of both sites divided by the total base pair size of the individual site giving the following: Valle Greene 100% band diversity and WPAFB 54.7%; this difference perhaps suggests a bacterial community that has out-competed the other communities for substrate availability, i.e., PCE thus optimizing its growth and forcing a reduced bacterial diversity. Lastly, further analysis in-between sections and intra-seasons are needed as the bacterial profiles differ significantly in both wetlands.

Appendix A

PowerSoil™ DNA Isolation Kit

Catalog No.	Quantity
12888-50	50 Preps
12888-100	100 Preps

Instruction Manual

Introduction

The PowerSoil™ DNA Isolation Kit* is comprised of a novel and proprietary method for isolating genomic DNA from environmental samples. The kit is intended for use with environmental samples containing a high humic acid content including difficult soil types such as compost, sediment, and manure. Other more common soil types have also been used successfully with this kit. The isolated DNA has a high level of purity allowing for more successful PCR amplification of organisms from the sample. PCR analysis has been performed to detect a variety of organisms including bacteria (e.g. *Bacillus subtilis, Bacillus anthracis*), fungi (e.g. yeasts, molds), algae and Actinomycetes (e.g. *Streptomyces*).

The PowerSoil DNA Isolation Kit distinguishes itself from MO BIO's UltraClean™ Soil DNA Isolation Kit with a **NEW** humic substance/brown color removal procedure. This new procedure is effective at removing PCR inhibitors from even the most difficult soil types.

Environmental samples are added to a bead beating tube for rapid and thorough homogenization. Cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the membrane. DNA is then ready for PCR analysis and other downstream applications.

This kit is for research purposes only. Not for diagnostic use.

***PATENT PENDING**

Version: 09142005

Required Equipment:

Microcentrifuge (10,000 x *g*)
Pipettors (50 µl - 500 µl)
Vortex
Vortex Adapter (MO BIO Catalog # 13000-V1)

Kit Contents

	Kit Catalog # 12888-50		Kit Catalog # 12888-100	
Component	**Catalog #**	**Amount**	**Catalog #**	**Amount**
PowerBead Tubes (contain 750µl solution)	12888-50-PBT	50	12888-100-PBT	100
PowerSoil Solution C1	12888-50-1	3.3 ml	12888-100-1	6.6 ml
PowerSoil Solution C2	12888-50-2	14 ml	12888-100-2	28 ml
PowerSoil Solution C3	12888-50-3	11 ml	12888-100-3	22 ml

PowerSoil Solution C4	12888-50-4	72 ml	12888-100-4	144 ml
PowerSoil Solution C5	12888-50-5	30 ml	12888-100-5	2 x 30 ml
PowerSoil Solution C6	12888-50-6	6 ml	12888-100-6	12 ml
PowerSoil Spin Filters (units in 2 ml tubes)	12888-50-SF	50	12888-100-SF	100
PowerSoil 2 ml Collection Tubes	12888-50-T	200	12888-100-T	400

Kit Storage
Kit reagents and components should be stored at room temperature (15-30°C).

Precautions

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

WARNING: Solution C5 contains ethanol. It is flammable.

IMPORTANT NOTE FOR USE: Make sure the 2 ml PowerBead Tubes rotate freely in your centrifuge without rubbing.

Experienced User Protocol
Please wear gloves at all times

1. To the PowerBead Tubes provided, add 0.25 gm of soil sample.
2. Gently vortex to mix.
3. **Check Solution C1.** If Solution C1 is precipitated, heat solution to 60°C until dissolved before use.
4. Add 60µl of Solution C1 and invert several times or vortex briefly.
5. Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog No. 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.
6. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds at room temperature. **CAUTION:** Be sure not to exceed 10,000 x *g* or tubes may break.
7. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect between 400 to 500µl of supernatant. Supernatant may still contain some soil particles.
8. Add 250µl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.
9. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*.
10. Avoiding the pellet, transfer up to, but no more than, 600µl of supernatant to a clean 2 ml Collection Tube (provided).
11. Add 200µl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes.
12. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*.
13. Avoiding the pellet, transfer up to, but no more than, 750µl of supernatant into a clean 2 ml Collection Tube (provided).
14. Add 1200µl of Solution C4 to the supernatant and vortex for 5 seconds.
15. Load approximately 675µl onto a Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow through and add an additional 675µl of supernatant to the Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Load the remaining supernatant onto the

Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. **Note**: A total of three loads for each sample processed are required.

16. Add 500µl of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x *g*.
17. Discard the flow through.
18. Centrifuge again at room temperature for 1 minute at 10,000 x *g*.
19. Carefully place Spin Filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the Spin Filter.
20. Add 100µl of Solution C6 to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog No. 17000-10).
21. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.
22. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required.

We recommend storing DNA frozen (-20° to -80°C). Solution C6 contains no EDTA. To concentrate the DNA see the Additional Information Section.

Detailed Protocol

Please wear gloves at all times

1. To the PowerBead Tubes provided, add 0.25 gm of soil sample.

   After your sample has been loaded into the PowerBead Tube, the next step is a homogenization and lysis procedure. The PowerBead Tube contains a buffer that will (a) help disperse the soil particles, (b) begin to dissolve humic acids and (c) protect nucleic acids from degradation.

2. Gently vortex to mix.

   Gentle vortexing mixes the components in the PowerBead Tube and begins to disperse the sample in the PowerBead Solution.

3. **Check Solution C1**. If Solution C1 is precipitated, heat solution to 60°C until the precipitate has dissolved before use.

   Solution C1 contains SDS and other disruption agents required for complete cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. If it gets cold, it will form a white precipitate in the bottle. Heating to 60°C will dissolve the SDS and will not harm the SDS or the other disruption agents. Solution C1 can be used while it is still warm.

4. Add 60µl of Solution C1 and invert several times or vortex briefly.

5. Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog No. 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.

   Note: *The vortexing step is critical for complete homogenization and cell lysis. Cells are lysed*

by a combination of chemical agents from steps 1-4 and mechanical shaking introduced at this step. By randomly shaking the beads in the presence of disruption agents, collision of the beads with microbial cells will cause the cells to break open.

The MO BIO Vortex Adapter is designed to be a simple platform to facilitate keeping the tubes tightly attached to the vortex. It should be noted that although you can attach tubes with tape, often the tape becomes loose and not all tubes will shake evenly or efficiently. This may lead to inconsistent results or lower yields. Therefore, the use of the MO BIO Vortex Adapter is a highly recommended and cost effective way to obtain maximum DNA yields.

6. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds at room temperature. **CAUTION:** Be sure not to exceed 10,000 x *g* or tubes may break.

7. Transfer the supernatant to a clean 2 ml Collection Tube (provided).

   Note: *Expect between 400 to 500µl of supernatant at this step. The exact recovered volume depends on the absorbency of your starting material and is not critical for the procedure to be effective. The supernatant may be dark in appearance and still contain some soil particles. The presence of carry over soil or a dark color in the mixture is expected in many soil types at this step. Subsequent steps in the protocol will remove both carry over soil and coloration of the mixture.*

8. Add 250µl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.

   Solution C2 contains a reagent to precipitate non-DNA organic and inorganic material including humic substances, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

9. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*.

10. Avoiding the pellet, transfer up to 600µl of supernatant to a clean 2 ml Collection Tube (provided).

    The pellet at this point contains non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellets.

11. Add 200µl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes.

    Solution C3 is a second reagent to precipitate additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

12. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*.

13. Transfer up to 750µl of supernatant to a clean 2 ml Collection Tube (provided).

The pellet at this point contains additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellets.

14. Add 1.2ml of Solution C4 to the supernatant (be careful solution doesn't exceed rim of tube) and vortex for 5 seconds.

    Solution C4 is a high concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, this will adjust the DNA solution salt concentrations to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the Spin Filters.

15. Load approximately 675µl onto a Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow through and add an additional 675µl of supernatant to the Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. **Note**: A total of three loads for each sample processed are required.
16. *DNA is selectively bound to the silica membrane in the Spin Filter device in the high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.*

17. Add 500µl of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x *g*.

    Solution C5 is an ethanol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the Spin Filter. This wash solution removes residual salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane.

18. Discard the flow through from the 2 ml Collection tube.

    This flow through fraction is just non-DNA organic and inorganic waste removed from the silica Spin Filter membrane by the ethanol wash solution.

19. Centrifuge at room temperature for 1 minute at 10,000 x *g*.

    This second spin removes residual Solution C5 (ethanol wash solution). It is critical to remove all traces of wash solution because the ethanol in Solution C5 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

20. Carefully place Spin Filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the Spin Filter.

    Note: *It is important to avoid any traces of the ethanol based wash solution.*

21. Add 100µl of Solution C6 to the center of the white filter membrane.

    Note: *Placing the Solution C6 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica Spin Filter membrane. As Solution C6*

(elution buffer) passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution C6 (10 mM Tris) which lacks salt.

Alternatively, sterile DNA-Free PCR Grade Water may be used for DNA elution from the silica Spin Filter membrane at this step (MO BIO Catalog No. 17000-10). Solution C6 contains no EDTA. If DNA degradation is a concern, Sterile TE may also be used instead of Solution C6 for elution of DNA from the Spin Filter.

22. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.

23. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required.

    We recommend storing DNA frozen (-20° to -80°C). Solution C6 does not contain any EDTA. To concentrate DNA see the Additional Information Section.

Additional Information

Amount of Soil to Process

This kit is designed to process 0.25 g of soil. For inquiries regarding the use of larger sample amounts, please contact technical support for suggestions. For wet soils, see information under "Wet Soil Sample" below.

Wet Soil Sample

If soil sample is high in water content, remove contents from PowerBead Tube (beads and solution) and transfer into another sterile microcentrifuge tube (not provided). Add soil sample to PowerBead Tube and centrifuge at room temperature for 30 seconds at 10,000 x g. Remove as much liquid as possible with a pipette tip. Add beads and bead solution back to PowerBead Tube and follow protocol starting at step 2.

If DNA Does Not Amplify

- Make sure to check DNA yields by gel electrophoresis or spectrophotometer reading. An excess amount of DNA will inhibit a PCR reaction.
- Diluting the template DNA should not be necessary with DNA isolated with the PowerSoil DNA Isolation Kit; however, it should still be attempted.
- If DNA will still not amplify after trying the steps above, then PCR optimization (changing reaction conditions and primer choice) may be needed.

Eluted DNA Sample Is Brown

We have not observed any coloration in DNAs isolated using the PowerSoil DNA Isolation kit. If you observe coloration in your samples, please contact technical support for suggestions.

Alternative Lysis Methods

- After adding Solution C1, vortex 3-4 seconds, then heat to 70°C for 5 minutes. Vortex 3-4 seconds. Heat another 5 minutes. Vortex 3-4 seconds. This alternative procedure will reduce shearing but may also reduce yield.
- If cells are difficult to lyse, a 10 minute incubation at 70°C, after adding Solution C1, can be performed. Follow by continuing with protocol step 5.

Concentrating the DNA

The final volume of eluted DNA will be 100µl. The DNA may be concentrated by adding 4µl of 5M NaCl and inverting 3-5 times to mix. Next, add 200µl of 100% cold ethanol and invert 3-5 times to mix. Centrifuge at 10,000 x g for 5 minutes at room temperature. Decant all liquid. Remove residual ethanol in a speed vac, dessicator, or air dry. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.

DNA Floats Out of Well When Loaded on a Gel

This usually occurs because residual Solution C5 remains in the final sample. Prevent this by being careful in step 19 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation (described in "Concentrating the DNA") is the best way to remove residual Solution C5.

Storing DNA

DNA is eluted in Solution C6 (10mM Tris) and must be stored at -20° to -80°C to prevent degradation. DNA can be eluted in TE without loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. DNA may also be eluted with sterile DNA-Free PCR Grade Water (MO BIO Catalog No. 17000-10).

Contact Information

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For the distributor nearest you, visit our web site at www.mobio.com/distributors

Appendix B

HotStarTaq PCR Protocol

PCR Protocol Using HotStarTaq DNA Polymerase

This protocol serves only as a guideline for PCR amplification. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be individually determined.

Notes:
- **HotStarTaq DNA Polymerase requires an activation step of 15 min at 95°C (see step 6 of this protocol).**
- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- If required, prepare a dNTP mix containing 10 mM of each dNTP. Store this mix in aliquots at –20°C.

1. **Thaw 10x PCR Buffer, dNTP mix, primer solutions, and 25 mM $MgCl_2$ (if required).**

   It is important to mix the solutions completely before use to avoid localized concentrations of salts.

2. **Prepare a master mix according to Table 1.**

   The master mix typically contains all the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should always be included. The optimal Mg^{2+} concentration should be determined empirically but in most cases a concentration of 1.5 mM, as provided in the 1x PCR Buffer, will produce satisfactory results.

Table 1. Reaction composition using HotstarTaq DNA Polymerase

Component	Volume/reaction	Final concentration
Master mix		
10x PCR Buffer*	10 µl	1x
25 mM $MgCl_2$	Variable, see Table 2	See Table 2
dNTP mix (10 mM of each)	2 µl	200 µM of each dNTP
Primer A	Variable	0.1–0.5 µM
Primer B	Variable	0.1–0.5 µM
HotStarTaq DNA Polymerase	0.5 µl	2.5 units/reaction
Distilled water	Variable	–
Template DNA		
Template DNA, added at step 4	Variable	≤1 µg/reaction
Total volume	**100 µl**	–

* *Contains 15 mM $MgCl_2$*

HotStarTaq PCR Protocol

Table 2. Final Mg^{2+} concentrations

Final Mg^{2+} concentration in reaction (mM):	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
Required volume of 25 mM $MgCl_2$ per reaction (µl):	0	2	4	6	8	10	12	14

3. **Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes.**
   Mix gently, e.g., by pipetting the master mix up and down a few times. It is not necessary to keep PCR tubes on ice as nonspecific DNA synthesis cannot occur at room temperature due to the inactive state of HotStarTaq DNA Polymerase.
4. **Add template DNA (≤1 µg/reaction) to the individual tubes containing the master mix.**
   For RT-PCR, add an aliquot from the reverse transcriptase reaction. This should not exceed 10% of the final PCR volume (see appendix, page 28).
5. **When using a thermal cycler with a heated lid, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 100 µl mineral oil.**
6. **Program the thermal cycler according to the manufacturer´s instructions.**
   Each PCR program must start with an initial heat activation step at 95°C for 15 min. A typical PCR cycling program is outlined below. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

			Additional comments
Initial activation step:	**15 min**	**95°C**	HotStarTaq DNA Polymerase is activated by this heating step.
3-step cycling			
Denaturation:	0.5–1 min	94°C	
Annealing:	0.5–1 min	50–68°C	Approximately 5°C below T_m of primers (see appendix, page 24).
Extension:	1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.
Number of cycles:	25–35		See appendix, page 25.
Final extension:	10 min	72°C	

7. **Place the PCR tubes in the thermal cycler and start the cycling program.**
   Note: After amplification, samples can be stored overnight at 2–8°C or at –20°C for longer storage.

Appendix C

Promega

Usage Information

Introduction

Restriction enzymes, also referred to as restriction endonucleases, are enzymes which recognize short, specific (often palindromic) DNA sequences. They cleave double-stranded DNA (dsDNA) at specific sites within or adjacent to their recognition sequences. Most restriction enzymes (REs) will not cut DNA that is **methylated** on one or both strands of their **recognition site**, although some require substrate methylation.

Each restriction enzyme has specific requirements to achieve optimal activity. Ideal storage and assay conditions favor the most activity and highest fidelity in a particular enzyme's function. Conditions such as temperature, pH, enzyme cofactor(s), salt composition and ionic strength affect enzyme activity and stability. Two buffers usually accompany each of Promega's restriction enzymes. One buffer is the optimal reaction buffer which may be from the **4-CORE® System** (Reaction Buffers A, B, C, D) or one of the other optimal buffers (Reaction Buffers E–L), and the other is the MULTI-CORE™ Buffer. The supplied optimal buffer always yields 100% activity for the enzyme it accompanies, and serves as the specific reaction buffer for individual digests with that enzyme. **The MULTI-CORE™ Buffer**, which is designed for broad compatibility with many REs, is provided with enzymes that have 25% or greater activity in this buffer. The MULTI-CORE™ Buffer is useful for multiple digests because it generally yields more activity for more enzyme combinations than any of the other buffers, but sometimes with a compromise in activity. Multiple digests using REs with significantly different buffer requirements may require a sequential reaction with the addition of RE buffer or salt before the second enzyme is used.

DNA Substrate Considerations

DNA substrates commonly used for restriction enzyme digestion include DNA from bacteriophage lambda, bacterial plasmid DNA and genomic DNA. Lambda DNA is a linear DNA form that is an industry standard for measuring and expressing unit activity for many restriction enzymes. Compared to linear DNA, intact supercoiled plasmid DNA (and DNAs with a large number of the target restriction site) require more units of enzyme (two- to tenfold) per microgram than the DNA used in the enzyme's activity assay.

PCR products and oligonucleotides are relatively small compared with DNA used for defining RE units. Therefore, when using these substrates in a restriction digest, it is essential to take into consideration the molar concentration of enzyme recognition sites and not just the mass of DNA. Also, some REs require flanking bases surrounding the core RE recognition site. This is problematic when it is necessary to cut an oligonucleotide or a fragment of DNA with an RE site near its end. When PCR cloning strategies include the use of primers containing an RE site, care is necessary in designing the primer with adequate DNA surrounding the core RE recognition sequence.

In addition to the form and original source of the DNA, the purity is another factor that must be considered. Depending on the purification method and the handling of the DNA, it may contain varying amounts of contaminants that affect restriction enzyme digestion and analysis. Contaminants may include other types of DNA, nucleases, salts and inhibitors of restriction enzymes. The effect of a contaminant on an RE digest is generally dose-dependent: i.e., the inhibitory effects will increase with the volume of DNA added to the restriction enzyme reaction. Relatively pure DNA is required for efficient restriction enzyme digestion. Contaminating nucleases are usually activated only after the addition of salts (e.g., restriction enzyme buffer) to the DNA solution. Therefore, appropriate control reactions should always be run in parallel with the restriction digest. Buffer solutions containing EDTA in low concentrations (1mM) are often used to protect DNA from nuclease degradation during storage, but the EDTA can interfere with restriction enzyme digestion if the final concentration of EDTA in the reaction is too high. This situation usually results when the concentration of the substrate DNA is low and it is necessary to use a large volume of DNA in the digest. In such cases, it is best to concentrate the DNA (e.g., by ethanol precipitation). The organic solvents, salts, detergents and chelating agents that are sometimes used during the purification of DNA can also interfere with restriction enzyme activity if they carry over into the final DNA solution. Dialysis and/or ethanol precipitation with 2.5M ammonium acetate (final concentration before adding ethanol) followed by drying and resuspension can remove many of these substances. While relatively pure DNA is required for efficient restriction enzyme digestion, addition of acetylated BSA to a final concentration of 0.1mg/ml can sometimes improve the quality and efficiency of enzyme assays containing impure DNA and we recommend that it be included in all digests.

Enzyme Storage, Handling and Use

Maintain the sterility of reagents used in the RE digest as well as any tools (e.g., tubes, pipette tips) used with those reagents. Restriction enzymes should be stored in a non-frost-free freezer, except for a brief period during use, when they should be kept on ice. The restriction enzyme is usually the last reagent added to a reaction, to ensure that it is not exposed to extreme conditions. When many similar digests are being prepared, it may be convenient to create premixes of common reagents.

Before assembling the restriction digest, thoroughly mix each component to be added to the reaction and then centrifuge the tubes of reagents briefly to collect the contents in the bottom of the tube. The reaction components should also be mixed after addition of the enzyme to the digest. While high salt buffers and glycerol-containing reagents are difficult to mix, all solutions containing restriction enzymes must be mixed gently to avoid inactivating the enzyme.

Setting up a Restriction Enzyme Digest

An analytical scale restriction enzyme digest is usually performed in a volume of 20μl on 0.2–1.5μg of substrate DNA, using a two- to tenfold excess of enzyme over DNA. If an unusually large volume of DNA or enzyme is used, aberrant results may occur and may or may not be readily recognized. The following is an example of a typical RE digest. In a sterile tube, assemble in order:

sterile, deionized water	16.3μl
RE 10X Buffer	2μl
Acetylated BSA, 10μg/μl	0.2μl
DNA, 1μg/μl	1μl

Mix by pipetting, then add:

Restriction Enzyme, 10u/μl	0.5μl
final volume	20μl

Mix gently by pipetting, close the tube and centrifuge for a few seconds in a microcentrifuge. Incubate at the optimum temperature for 1–4 hours.

Add 4μl of 6X loading buffer and proceed to gel analysis. Note that overnight digests are usually unnecessary and may result in degradation of the DNA.

Experimental Controls

Experimental controls are necessary to identify, understand and explain problems or inconsistencies in results. The following controls are commonly used in parallel with RE digests: (i) uncut experimental DNA, (ii) digest of commercially supplied control DNA, (iii) no-enzyme "mock" digest, (iv) 1 or 2 different size markers in more than one lane per gel (i.e., different locations).

Appendix D

Promega

Wizard® SV Gel and PCR Clean-Up System

All technical literature is available on the Internet at www.promega.com/tbs
Please visit the web site to verify that you are using the most current version of this Technical Bulletin. Please contact Promega Technical Services if you have questions on use of this system. E-mail techserv@promega.com.

I. Description

The Wizard® SV Gel and PCR Clean-Up System is designed to extract and purify DNA fragments of 100bp to 10kb from standard or low-melt agarose gels in either Tris acetate (TAE) or Tris borate (TBE), or to purify PCR products directly from a PCR amplification[(a)]. Up to 95% recovery is achieved depending upon the DNA fragment size (see Table 1). PCR products are commonly purified to remove excess nucleotides and primers. This membrane-based system, which can bind up to 40µg DNA, allows recovery of isolated DNA fragments or PCR products in as little as 20 minutes, depending on the number of samples processed and the protocol used. The purified DNA can be used for automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion or in vitro transcription/translation without further manipulation.

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Part# TB308

Promega

The Wizard® SV Gel and PCR Clean-Up System is based on the ability of DNA to bind to silica membranes in the presence of chaotropic salts. After electrophoresis to separate the DNA fragments, the band(s) of interest is excised and dissolved in the presence of guanidine isothiocyanate (Membrane Binding Solution). Alternatively, after amplification, an aliquot of PCR reaction is added to the Membrane Binding Solution and directly purified. The system allows a choice of methods for isolation of DNA from the dissolved agarose gel slice or PCR reaction. DNA can be isolated using microcentrifugation to force the dissolved gel slice or PCR reaction through the membrane while simultaneously binding the DNA on the surface of the silica (Section V.A). After washing the isolated DNA fragment or PCR product, the DNA is eluted in water. Another option is pulling the dissolved gel or PCR reaction through the SV Minicolumn and washing the DNA fragment using vacuum pressure (Section V.B). The Vacuum Adapters allow the use of a vacuum manifold (e.g., Vac-Man® Laboratory Vacuum Manifold, 20-sample capacity [Cat.# A7231], or Vac-Man® Jr. Laboratory Vacuum Manifold, 2-sample capacity [Cat.# A7660]). The Vacuum Adapters (Cat. # A1331) are only supplied with Cat.# A9280, Wizard® SV Gel and PCR Clean-Up System, 10 preps, but may be purchased separately.

The Wizard® SV Gel and PCR Clean-Up System can be used with linear DNA fragments, supercoiled plasmid DNA, or single-stranded linear or circular DNA. Expected yields with single-stranded DNA are lower than for double-stranded DNA.

Table 1. Percent Recovery Versus Double-Stranded DNA Fragment Size. PCR products (55–1,000bp), linearized pGEM®-3Zf(+) plasmid (3,199bp), or Lambda *Hind* III fragments (9,416bp and 23,130bp) were purified in triplicate from a 1% agarose gel slice in 1X TAE buffer and quantified by ethidium bromide staining.

DNA Fragment Size	Percent Recovery
55bp	26%
70bp	39%
85bp	55%
100bp	84%
500bp	89%
1,000bp	92%
3,199bp	95%
9,416bp	95%
23,130bp	47%

II. Product Components

Product	Size	Cat. #
Wizard® SV Gel and PCR Clean-Up System	10 preps	A9280

For Laboratory Use. Each system contains sufficient reagents for 10 purifications.
Includes:

- 4ml Membrane Binding Solution
- 3ml Membrane Wash Solution (concentrated)
- 1.25ml Nuclease-Free Water
- 10 Wizard® SV Minicolumns
- 10 Collection Tubes (2ml)
- 5 Vacuum Adapters
- 1 Protocol

Product	Size	Cat. #
Wizard® SV Gel and PCR Clean-Up System	50 preps	A9281

For Laboratory Use. Each system contains sufficient reagents for 50 purifications.
Includes:

- 20ml Membrane Binding Solution
- 15ml Membrane Wash Solution (concentrated)
- 3.75ml Nuclease-Free Water
- 50 Wizard® SV Minicolumns
- 50 Collection Tubes (2ml)
- 1 Protocol

Product	Size	Cat. #
Wizard® SV Gel and PCR Clean-Up System	250 preps	A9282

For Laboratory Use. Each system contains sufficient reagents for 250 purifications.
Includes:

- 100ml Membrane Binding Solution
- 75ml Membrane Wash Solution (concentrated)
- 13ml Nuclease-Free Water
- 250 Wizard® SV Minicolumns
- 250 Collection Tubes (2ml)
- 1 Protocol

Storage Conditions: Store all components at room temperature (22–25°C). No refrigeration is required. Keep Membrane Binding Solution protected from light. See expiration date on product label.

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III. General Considerations

Agarose, a linear polymer extracted from seaweed, is commonly used for electrophoretic separation of nucleic acids. Standard agarose melts at 87–89°C and solidifies at 36–39°C. In low-melt agarose, hydroxyethyl groups have been introduced into the polysaccharide chain, resulting in an agarose that both melts and solidifies at much lower temperatures (65°C and 24–28°C, respectively). Low-melt agarose is often used for applications that require recovery of intact DNA fragments from the gel after electrophoresis. The Wizard® SV Gel and PCR Clean-Up System can be used to recover DNA from either standard or low-melt agarose gels with no changes to the protocol or differences in recovery (Section V).

Standard safety apparel should be worn, especially when handling ethidium bromide-stained agarose gels. This includes gloves and a UV-blocking face shield to protect the eyes and face from UV light. When excising the gel band, work quickly to minimize personal exposure to UV light and to minimize nicking of the DNA (1–4).

The Wizard® SV Gel and PCR Clean-Up System is compatible with PCR products generated using a variety of amplification enzymes, buffers or PCR-enhancing additives. Mineral oil does not interfere with purification.

IV. Gel Slice and PCR Product Preparation

Materials to Be Supplied by the User

(Solution compositions are provided in Section VIII.A.)

- 1.5ml microcentrifuge tubes
- ethanol (95%)
- Vacuum Adapters (Cat.# A1331; only for vacuum purification)
- agarose gel (standard or low-melt; only for gel purification)
- 1X TAE or TBE electrophoresis buffer (only for gel purification)
- 50–65°C heating block (only for gel purification)

A. Preparing the Membrane Wash Solution

Add the indicated volume of 95% ethanol to the Membrane Wash Solution prior to beginning the procedure (see Table 2). Mark the bottle label to record that this addition was made. Tightly close the bottle cap after each use to prevent evaporation.

Table 2. Volume of 95% Ethanol to Add to Membrane Wash Solution for Each System Size.

System Size	Part Number of Membrane Wash Solution	Volume of 95% Ethanol
10 preps	A929A	15ml
50 preps	A929B	75ml
250 preps	A929C	375ml

Promega

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C. Processing PCR Reactions

1. Amplify target of choice using standard amplification conditions.
2. Add an equal volume of Membrane Binding Solution to the PCR reaction (see Notes 1–4 below).
3. To purify the DNA using a microcentrifuge, proceed to Section V.A.
   To purify the DNA using a vacuum manifold, proceed to Section V.B.

Notes:

1. The maximal capacity of a single SV Minicolumn is approximately 1ml of PCR reaction added to 1ml Membrane Binding Solution (2ml total). For PCR volumes >350µl, continue to pass the sample through the column until all of the sample has been processed.
2. The maximum binding capacity is approximately 40µg per column, and as little as 10ng has been successfully purified.
3. Mineral oil does not interfere with purification.
4. For amplification reactions that do not produce a single product or where amplification has been inefficient and there is highly visible primer dimer, gel purification of the band of interest is recommended. Alternatively, an 80% ethanol wash solution can be substituted for the supplied Membrane Wash Solution to reduce primer-dimer carryover.

V. DNA Purification

Prepare the gel slice or PCR product as described in Section IV. Use either the centrifugation procedure (Section V.A) or the vacuum procedure (Section V.B) to recover the DNA from the dissolved gel slice or PCR reaction. After the procedure is completed, the DNA may be used in downstream applications.

A. DNA Purification by Centrifugation

1. Place one SV Minicolumn in a Collection Tube for each dissolved gel slice or PCR reaction.
2. Transfer the dissolved gel mixture or prepared PCR product to the SV Minicolumn assembly and incubate for 1 minute at room temperature.
3. Centrifuge the SV Minicolumn assembly in a microcentrifuge at 16,000 × *g* (14,000rpm) for 1 minute. Remove the SV Minicolumn from the Spin Column assembly and discard the liquid in the Collection Tube. Return the SV Minicolumn to the Collection Tube.

Note: Failure to spin at 16,000 × *g* (14,000rpm) can result in reduced yield.

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4. Wash the column by adding 700µl of Membrane Wash Solution, previously diluted with 95% ethanol (see Section IV.A), to the SV Minicolumn. Centrifuge the SV Minicolumn assembly for 1 minute at 16,000 × *g* (14,000rpm). Empty the Collection Tube as before and place the SV Minicolumn back in the Collection Tube. Repeat the wash with 500µl of Membrane Wash Solution and centrifuge the SV Minicolumn assembly for 5 minutes at 16,000 × *g*.
5. Remove the SV Minicolumn assembly from the centrifuge, being careful not to wet the bottom of the column with the flowthrough. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the micro-centrifuge lid open (or off) to allow evaporation of any residual ethanol.
6. Carefully transfer the SV Minicolumn to a clean 1.5ml microcentrifuge tube. Apply 50µl of Nuclease-Free Water directly to the center of the column without touching the membrane with the pipette tip. Incubate at room temperature for 1 minute. Centrifuge for 1 minute at 16,000 × *g* (14,000rpm).
7. Discard the SV Minicolumn and store the microcentrifuge tube containing the eluted DNA at 4°C or –20°C.

Note: The volume of the eluted DNA will be approximately 42–47µl. If the DNA needs to be further concentrated, perform an ethanol precipitation. Alternatively, the DNA may be eluted in as little as 15µl of Nuclease-Free Water without significant reduction in yield. If using an elution volume of 15µl, verify that the membrane is completely covered with Nuclease-Free Water before centrifugation. Elution volumes less than 15µl are not recommended (see Table 3).

B. DNA Purification by Vacuum

1. Attach one Vacuum Adapter with a Luer-Lok® fitting to one port of the manifold (e.g., Vac-Man® or Vac-Man® Jr. Laboratory Vacuum Manifold) for each dissolved gel slice or PCR reaction. Insert SV Minicolumn into the Vacuum Adapter until it fits snugly in place.
2. Transfer the dissolved gel mixture or PCR reaction to the SV Minicolumn and incubate for 1 minute at room temperature. Apply a vacuum to pull the liquid completely through the SV Minicolumn.

Note: The minimum vacuum pressure is 15 inches of mercury. See the table to the right for comparison of inches of Hg to other pressure measurements.

1 Inch Hg	15 Inches Hg
3.386kPa	50.8kPa
25.4Torr	381Torr
0.0334atm	0.501atm
0.491psi	7.37psi
2.54cm Hg	38.1cm Hg
33.86mbar	508mbar

Appendix E

Running the agarose gel

1. **<u>Mix TAE solution</u>**
   a. Make a Tris-acetic acid-disodium EDTA (TAE) solution. This solution comes from WSU at a 50X concentration. Dilute to a 1X concentration. The formula for this is

$$C_1 * V_1 = C_2 * V_2$$

   b. For this requirement, we would like to get 500mL of TAE at a concentration of 1X so we plug in the known values to get the volume of TAE @ 50X concentration and then subtract that from the overall 500mL to get the amount of distilled water.

$$C_1 = 1X$$
$$V_1 = 1000mL$$
$$C_2 = 50X$$
$$V_2 = ?$$

   c. Plug in known values and solve for V_2:

$$\frac{(1X) * (1000mL)}{(50X)} = 20mL = \mathrm{V}_2$$

   d. Subtract the V_2 from the overall solution that we want to make and that gives the amount of distilled water that we need which to make a 1X solution of 1000mL. This means we need 980mL of DI and 20mL of TAE 50X.

2. **Mixing Agarose Gel** (for small gel box; adjust by a factor of 1.5 for large tray).
   a. Make a 70mL of an 1% Agarose solution.
   b. Mix 0.70g of Agarose into 70mL of TAE 1X solution
   c. Mix and place into microwave
   d. Microwave on high for about 1.5 to 2.0 minutes to bring solution to boil.
   e. Once Agarose is dissolved remove from microwave and allow to cool to touch.
   f. Add $70\mu L$ of ETBR 1000X and swirl
      i. **General Information:** Ethidium Bromide (EtBr) is a commonly used stain for the visualization of nucleic acids in agrose gels. It is widely used by scientists due to its high sensitivity, rapid staining and very inexpensive price. While it is not specifically regulated as a hazardous waste, the mutagenic properties may present a hazard if it is not managed properly in the laboratory.
      ii. **Personal Protection:** When handling EtBr always wear a lab coat, nitrile gloves, and chemical splash goggles. Proper skin and eye protection are needed when a ultraviolet (UV) light source is used while working with EtBr. Avoid exposing unprotected skin and eyes to intense UV sources. Wear a face shield if UV source is pointing upwards. When working with a UV source for a long time, wrap up lab coat sleeves with tape or other means where the wrist could be exposed.

iii. **Disposal of EtBr**: Electrophoresis Gels: Trace amounts of EtBr (less than 0.1%) in electrophoresis gels do not pose a serious hazard so they can be discarded in the trash if properly bagged and secured. If the gels contain more than 0.1% EtBr they should be placed in an appropriate container for hazardous waste disposal. Environmental Health and Safety (EH&S) has a variety of containers that are available to collect and dispose of gels.

g. Place comb in gel box
h. Add the 70mL of solution to the Gel Bed and allow hardening approximately 30 minutes.
i. Once gel is hardened, remove comb from gel bed.
j. Extract gel bed from gel box and rotate 90 degrees so that wells formed by the comb are opposite the red (Pos) leads.
k. Fill Gel box with 1X TAE until both sides of gel box overflow and cover the hardened gel.

3. Prepare PCR Samples for Gel

a. Take PCR sample and remove 3microliters into a autoclaved Eppendorf tube
b. 17 to 18.5 μL of distilled water depending on buffer concentrations of 6X and 10X, respectively.
c. $2.4\mu L$ or $4\mu L$ of 10X or 6X buffer, respectively (does not have to be refrigerated).

4. Load DNA into Wells and Run GEL

a. Add $6\mu L$ of 1kb DNA Ladder into the 1st well (Toward the Black (-) Lead)
b. Add $24\mu L$ of PCR samples for Gel prepared in step 3
c. Attach colored leads to matching receptors on Gel box and power source.
d. Turn on power source and allow to run until there is a clear separation (Approximately 45 minutes).

5. Imaging of GEL via Kodak Gel Logic 200

Appendix F

Steps in sample processing and analysis

I. Sample Collection and Preparation
   a. Core collection
      i. Once per season once, per site from depth of 0 to 9, 9 to 27, and 27 to 45 in
      ii. Split cores into sections
   b. Core sampling
      i. Two, 10 gram(g) samples, aseptically removed from each of 3 sections, one from each depth
      ii. Two sites, so a total of 6 sections to be sampled, therefore a total of 12- 10 g samples will be homogenized
      iii. Prepare four 0.5 g sub-samples from each 10 g sample, for a total of 48- 0.5 g samples.
      iv. Store 0.5 g samples at 4°C; store remainder of 10 g samples at -20°C.

   c. DNA extractions from soil
      i. Perform DNA extractions on the 0.25 g sub-samples from each original 0.5g samples; 48 extractions total. Archive additional 0.25g at 4°C as a backup sample in the event procedures do not yield bacterial DNA
      ii. Include two buffer negative controls; brings total up to 50.
      iii. Store all DNA extracts at -80°C.

II. PCR
   a. 1-3 weeks.
      i. Design and order primers in accordance with manufactures recommendations (INVITROGEN Qiagen protocol for soil PCR analysis); procedure optimized by Wright State University
      ii. Purify control DNA for amplification optimization (Qiagen protocol for soil PCR analysis); procedure optimized by Wright State University
      iii. Perform reactions with positive controls to make sure primers amplify a reductive dehalogenase
      iv. Verify primer specificity
   b. Internal positive control PCR reactions
      i. An endogenous target is amplified to screen for inhibitory contaminants in the DNA extracts
      ii. Inhibited extracts can be diluted and reassayed
   c. Sample amplification
      i. Will use two of the four extracts prepared above, so a total of 24 extracts.
         1. Will use one of the negative controls, brings total samples to be amplified up to 25.
         2. Perform two PCR reactions on each sample, total of 50 PCR reactions.
      ii. Validation of PCR
         1. Screen all reactions for products by gel electrophoresis.
         2. Pool the successful PCR reactions for each sample, total of 25 pools.

III. Electrophoresis
   a. Run each 0.25g PCR vial samples in accordance with HotStarTaq Master Mix protocol (see appendix C).
   b. Annotate positive results and run positive samples again for validation.
   c. Capture electrophoresis image in accordance with Kodak 1D Gel Logic 200 Image Imaging System manual Store all positive results at -80°C

IV. RFLP analysis
   a. Purify all PCR samples in accordance with ProOmega protocol (see appendix D)
   b. Digestion of verified bacterial rDNA with Rsa1 in accordance with ProOmega protocol (see appendix E).
   c. Run digested samples on a 2.5 % agarose gel for 3.5 hours

V. Data analysis
   a. Analysis will consist of comparison of bp alignment between wetlands and across the different depths, and
   b. May be able to correlate abundance/presence of specific dehalogenase with GC data for the contaminants.
   c. May be able to develop a phylogenic tree.

Appendix G: Acronyms

AFIT: Air Force Institute of Technology
ATSDR: Agency for Toxic Substances and Disease Registry
CAH: Chlorinated aliphatic hydrocarbon
CERCLA: Comprehensive Environmental Response, Compensation, and Liability Act
DCE: dichloroethylene
DNA: deoxyribonucleic acid
DNAPL: dense nonaqueous-phase liquid
EPA: Environmental Protection Agency
MCL: Maximum contaminant level
MMO Methane monooxygenase
NPL: National Priority List
NRC: National Research Council
PCE: tetrachloroethylene
PCR: Polymerase Chain Reaction
RFLP: Restriction Fragment Length Polymorphism
RCRA Resource Conservation and Recovery Act
TCA: trichloroethane
TCE: trichloroethylene
USDHHS: U.S. Department of Health and Human Services
VC: vinyl chloride
VOC: volatile organic compound
WPAFB: Wright-Patterson Air Force Base
WSU Wright State University

Appendix H: Research Flowchart

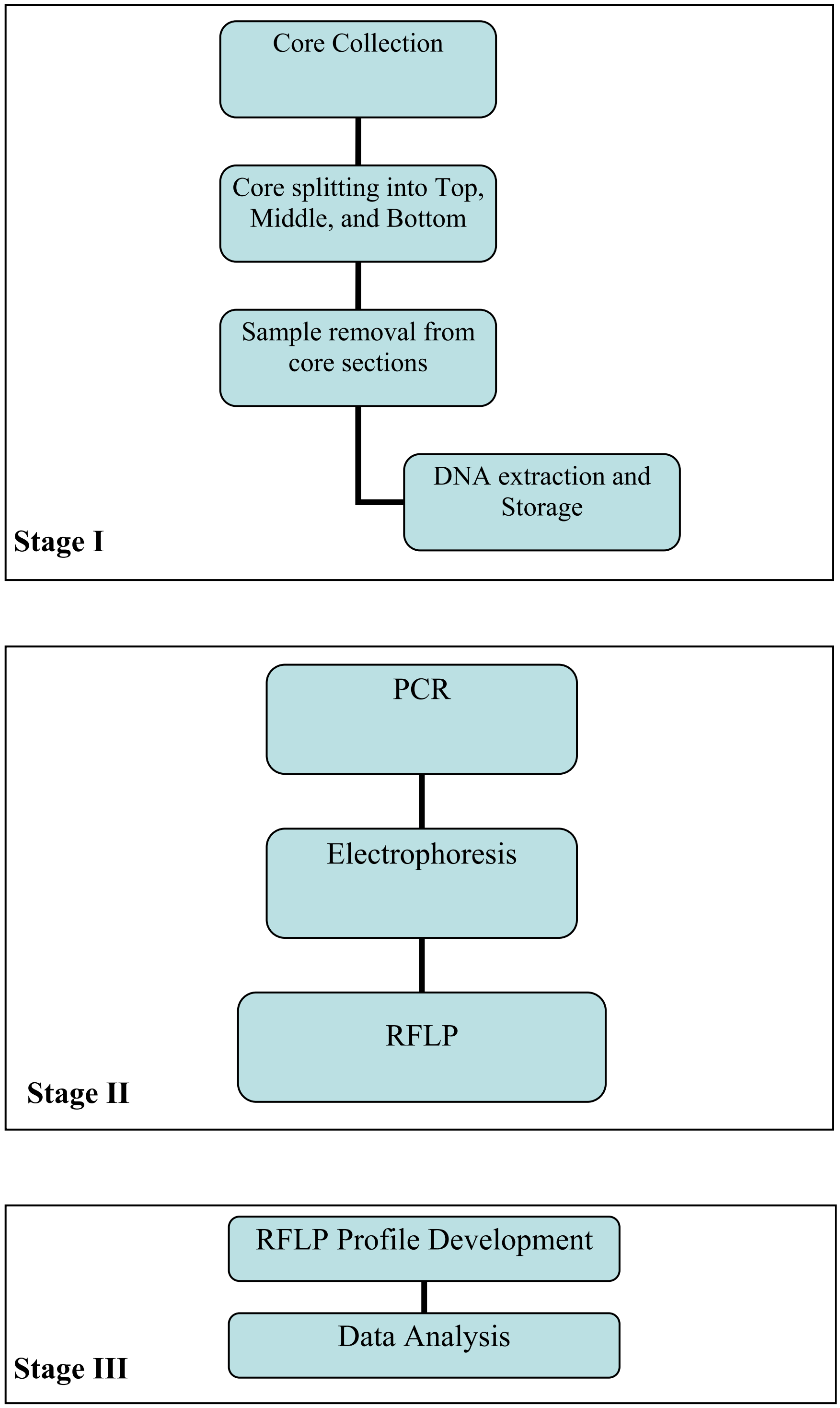
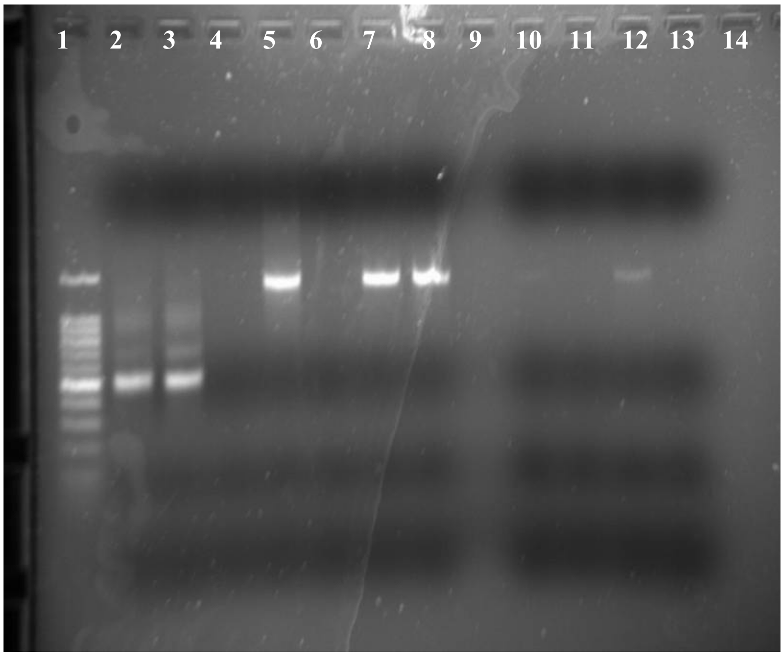


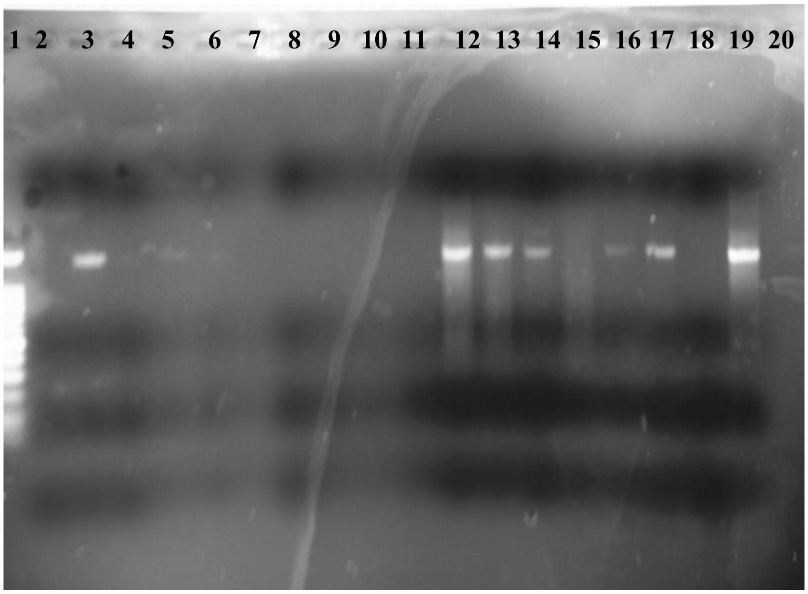
Fig 17 Steps in research completion

Fig 18b
PCR products using universal primers (F)E8f, (F)AU1406r, E8f and E533R directly extracted from soil collected from core 4 and wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1 hr with 10.5ul of DNA. Taken 11 Aug, 05. Note sample correspond to slide I.D. 7 on table 6



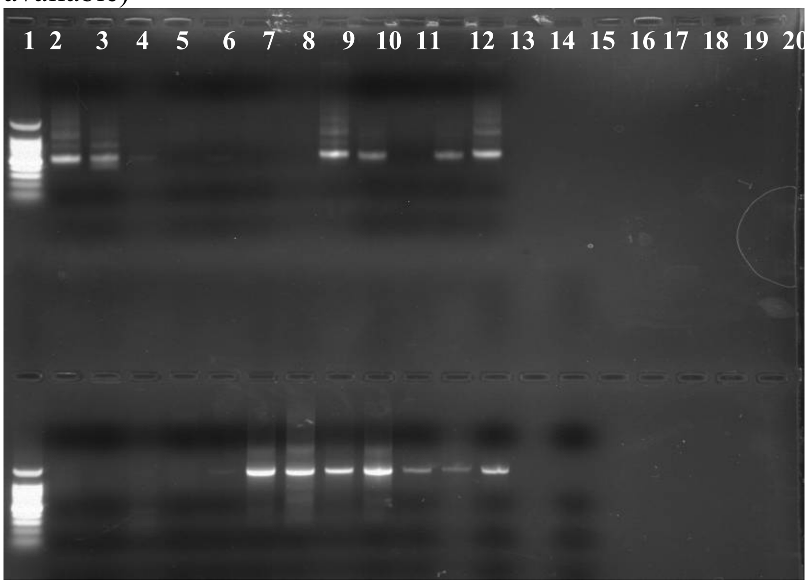
Lane 1- 1k bp DNA ladder
Lane 2- sample 40, core 4 section 3
Lane 3- sample 41, core 4 section 3
Lane 4- sample 42, core 4 section 1
Lane 5- sample 43, core 4 section 1
Lane 6- sample 44, core 4 section 2
Lane 7- sample 45, core 4 section 2
Lane 8- sample 46, core 4 section 2
Lane 9- sample 47, core 4 section 3
Lane 10-sample 48, core 4 section 3
Lane 11- sample 49, core 5 section 3
Lane 12- sample 50, core 5 section 3
Lane 13- control

Fig 18c
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 and 5 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 10.5ul of DNA. Taken 12 Aug, 05. Note sample correspond to slide I.D. 8 on table 6



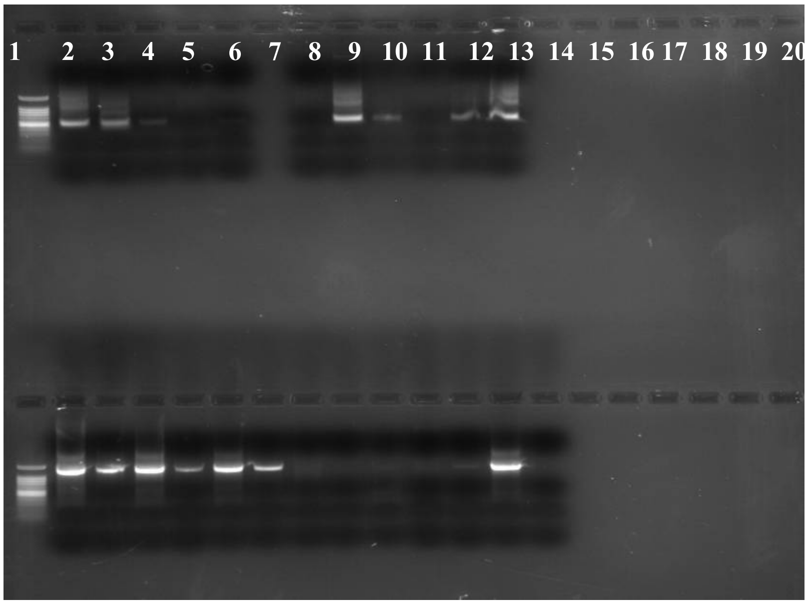
Lane 1- 1k bp DNA ladder
Lane 2- sample 52, core 4 section 1
Lane 3- sample 53, core 4 section 1
Lane 4- sample 54, core 4 section 1
Lane 5- sample 55, core 4 section 1
Lane 6- sample 56, core 4 section 2
Lane 7- sample 57, core 4 section 2
Lane 8- sample 58, core 4 section 2
Lane 9- sample 59, core 4 section 3
Lane 10-sample 60, core 4 section 3
Lane 11- blank
Lane 12- sample 62, core 5 section 1
Lane 13- sample 63, core 5 section 1
Lane 14- sample 64, core 5 section 1
Lane 15- sample 65, core 5 section 1
Lane 16- sample 66, core 5 section 2
Lane 17- sample 67, core 5 section 2
Lane 18- sample 68, core 5 section 3
Lane 19- sample 69, core 5 section 3
Lane 20- control

Fig 18d
PCR products using universal primers (F)E8f, (F)AU1406r, E8f and E533R directly extracted from soil collected from core 6 and7 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 10.5ul of DNA. Taken 12 Sept, 05. Note sample correspond to slide I.D. 9 on table 6. Top half of gel depicts greenhouse samples (sample I.D. not available)



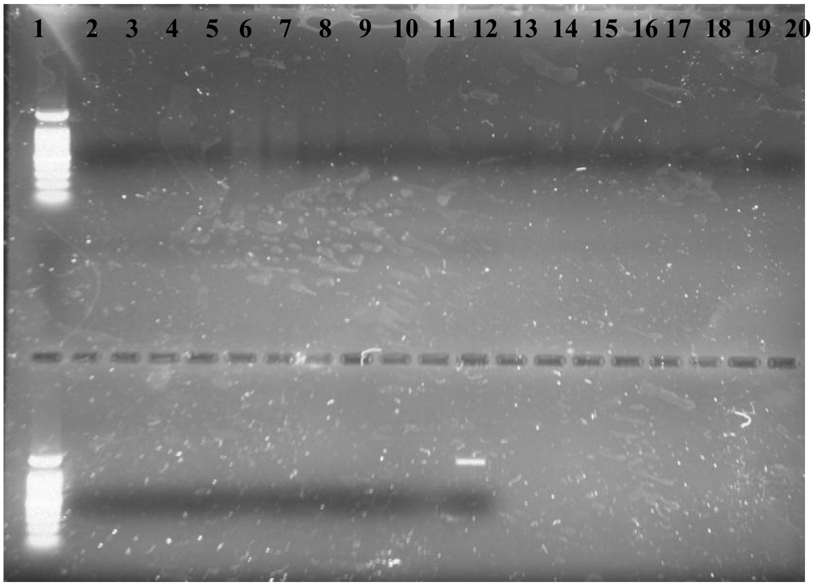
Lane 1- 1k bp DNA ladder
Lane 2- sample 70, core 6 section 1
Lane 3- sample 71, core 6 section 1
Lane 4- sample 72, core 6 section 2
Lane 5- sample 73, core 6 section 2
Lane 6- sample 74, core 6 section 3
Lane 7- sample 75, core 6 section 3
Lane 8- sample 76, core 7 section 1
Lane 9- sample 77, core 7 section 1
Lane 10- sample 78, core 7 section 2
Lane 11- sample 79, core 7 section 2
Lane 12- sample 80, core 7 section 3
Lane 13- sample 81, core 7 section 3
Lane 20- control

Fig 18e
PCR products using universal primers (F)E8f, (F)AU1406r, E8f and E533R directly extracted from soil collected from core 6 and7 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 10.5ul of DNA. Taken 12 Sept, 05. Note sample correspond to slide I.D. 10 on table 6. Top half of gel depicts greenhouse samples (sample I.D. not available)



Lane 1- 1k bp DNA ladder
Lane 2- sample 83, core 6 section 1
Lane 3- sample 84, core 6 section 1
Lane 4- sample 85, core 6 section 2
Lane 5- sample 86, core 6 section 2
Lane 6- sample 87, core 6 section 3
Lane 7- sample 88, core 6 section 3
Lane 8- sample 89, core 7 section 1
Lane 9- sample 90, core 7 section 1
Lane 10- sample 91, core 7 section 2
Lane 11- sample 92, core 7 section 2
Lane 12- sample 93, core 7 section 3
Lane 13- sample 94, core 7 section 3
Lane 20- control

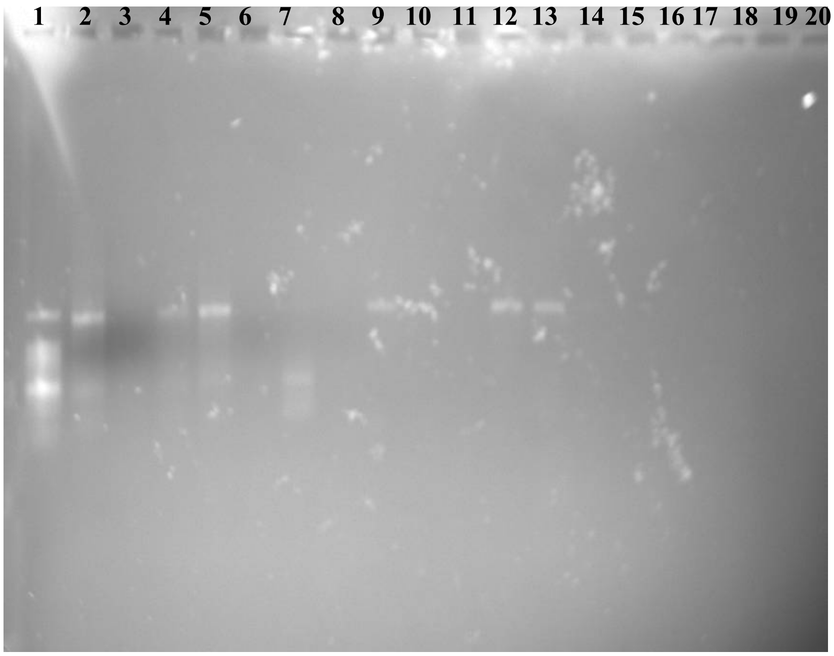
Fig 18f
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 10.5 and 5.5ul of DNA. Taken 20 Sep, 05. Note sample correspond to slide I.D. 11 on table 6



Lane 1- 1k bp DNA ladder
Lane 2- sample 96, core 4 section 1
Lane 3- sample 97, core 4 section 2
Lane 4- sample 98, core 4 section 3
Lane 5- sample 99, core 4 section 3
Lane 6- sample 100, core 5 section 1
Lane 7- sample 101, core 5 section 1
Lane 8- sample 102, core 5 section 1
Lane 9- sample 103, core 5 section 1
Lane 10- sample 104, core 5 section 2
Lane 11- sample 105, core 5 section 2
Lane 12- sample 106, core 5 section 3
Lane 13- sample 107, core 5 section 3
Lane 14- sample 108, core 1 section 1
Lane 15- sample 109, core 1 section 1
Lane 16- sample 110, core 1 section 2
Lane 17- sample 111, core 1 section 2
Lane 18- sample 112, core 1 section 3
Lane 19- sample 113, core 1 section 3
Lane 20- sample 114, core 2 section 1

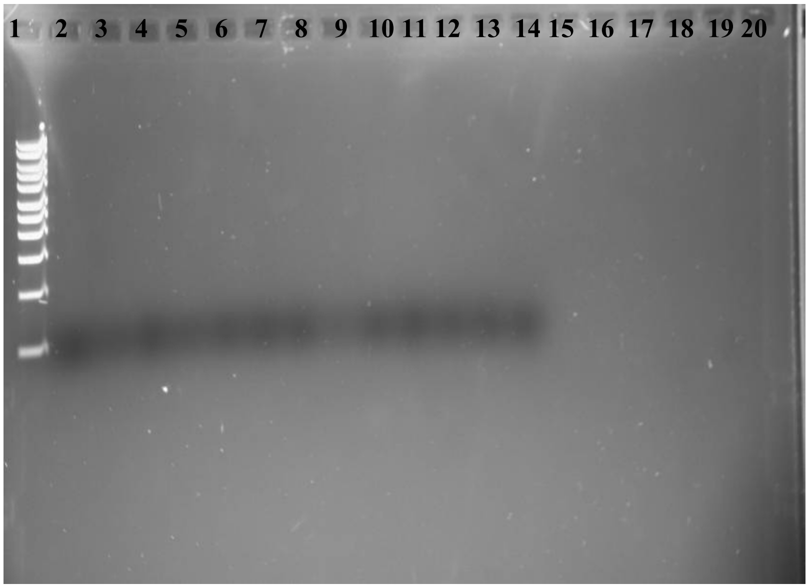
Lane 1- 1k bp DNA ladder (bottom row)
Lane 2- sample 115, core 2 section 1
Lane 3- sample 116, core 2 section 2
Lane 4- sample 117, core 2 section 2
Lane 5- sample 117, core 2 section 3
Lane 6- sample 118, core 2 section 3
Lane 8- sample 119, core 7 section 1
Lane 9- sample 120, core 7 section 1
Lane 10- sample 121, core 7 section 2
Lane 11- sample 122, core 7 section 2
Lane 12- sample 123, core 7 section 3
Lane 13- control

Fig 18g
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 10.5 and 5.0ul of DNA. Taken 18 Oct, 05. Note sample correspond to slide I.D. 15 on table 6



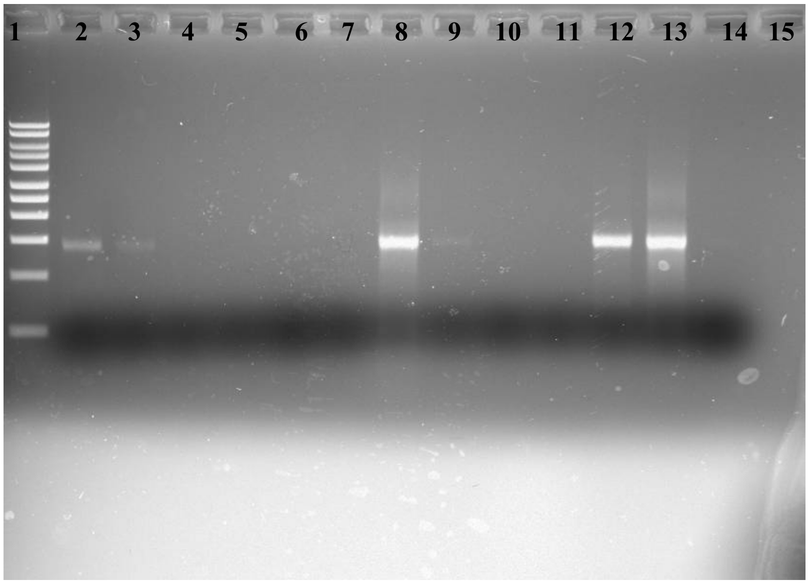
Lane 1- 1k bp DNA ladder
Lane 2- sample 185, core 2 section 1
Lane 3- sample 186, core 2 section 1
Lane 4- sample 187, core 2 section 2
Lane 5- sample 188, core 2 section 2
Lane 6- sample 189, core 2 section 3
Lane 7- sample 190, core 2 section 3
Lane 8- sample 191, core 5 section 1
Lane 9- sample 192, core 5 section 1
Lane 10- sample 193, core 5 section 1
Lane 11- sample 194, core 5 section 2
Lane 12- sample 195, core 5 section 2
Lane 13- sample 196, core 5 section 3
Lane 13- sample 197, core 7 section 3
Lane 20- control

Fig 18h
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 7ul of DNA. Taken 24 Oct, 05. Note sample correspond to slide I.D. 16 on table 6



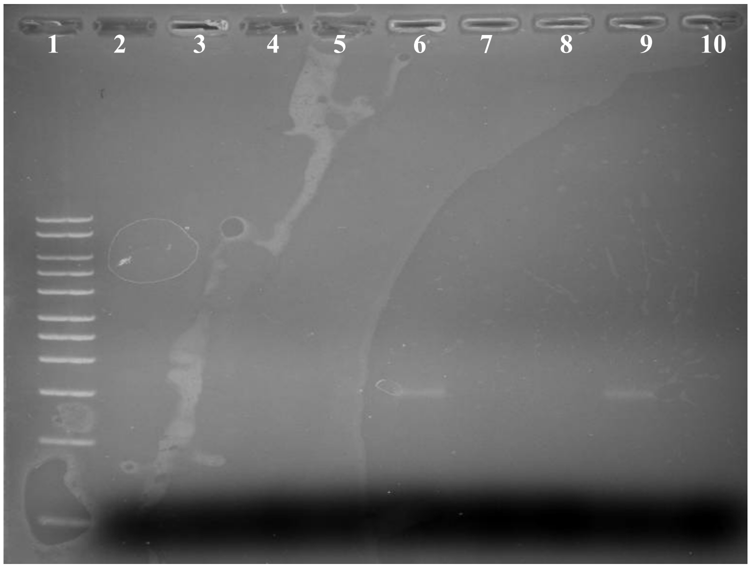
Lane 1- 1k bp DNA ladder
Lane 2- sample 198, core 6 section 1
Lane 3- sample 199, core 6 section 1
Lane 4- sample 200, core 6 section 2
Lane 5- sample 202, core 6 section 2
Lane 6- sample 203, core 6 section 3
Lane 7- sample 204, core 6 section 3
Lane 8- sample 205, core 1 section 1
Lane 9- sample 206, core 1 section 1
Lane 10- sample 207, core1 section 1
Lane 11- sample 208, core 1 section 2
Lane 12- sample 209, core 1 section 2
Lane 13- sample 210, core 1 section 3
Lane 13- sample 211, core 1 section 3
Lane 20- control

Fig 18i
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 3ul of DNA. Taken 25 Oct, 05. Note sample correspond to slide I.D. 17 on table 6



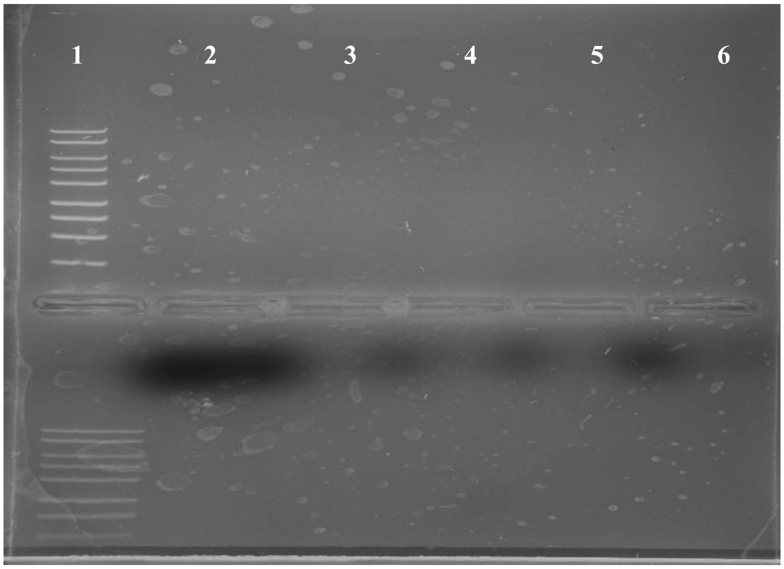
Lane 1- 1k bp DNA ladder
Lane 2- sample 211, core 6 section 1
Lane 3- sample 212, core 6 section 1
Lane 4- sample 213, core 6 section 2
Lane 5- sample 214, core 6 section 2
Lane 6- sample 215, core 6 section 3
Lane 7- sample 216, core 6 section 3
Lane 8- sample 217, core 1 section 1
Lane 9- sample 218, core 1 section 1
Lane 10- sample 219, core1 section 1
Lane 11- sample 220, core 1 section 2
Lane 12- sample 221, core 1 section 2
Lane 13- sample 222, core 1 section 3
Lane 14- sample 223, core 1 section 3
Lane 15- control

Fig 18i
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 5ul of DNA. Taken 14 Nov, 05. Note sample correspond to slide I.D. 23 on table 6



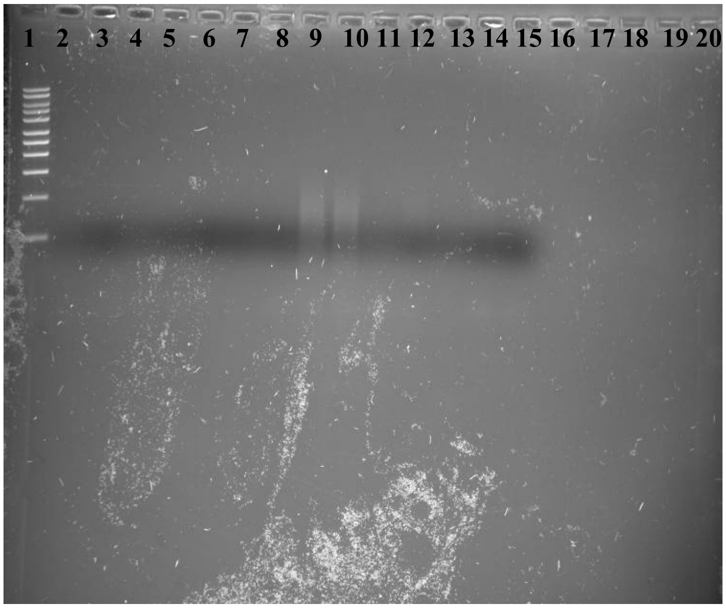
Lane 1- 10k bp DNA ladder
Lane 2- sample 290, core 9 section 1
Lane 3- sample 291, core 9 section 1
Lane 4- blank
Lane 5- sample 293, core 9 section 1
Lane 6- sample 294, core 9 section 1
Lane 7- blank
Lane 8- sample 296, core 9 section 1
Lane 9- sample 297, core 9 section 1
Lane 10- control

Fig 18j
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 5ul of DNA. Taken 15 Nov, 05. Note sample correspond to slide I.D. 24 on table 6



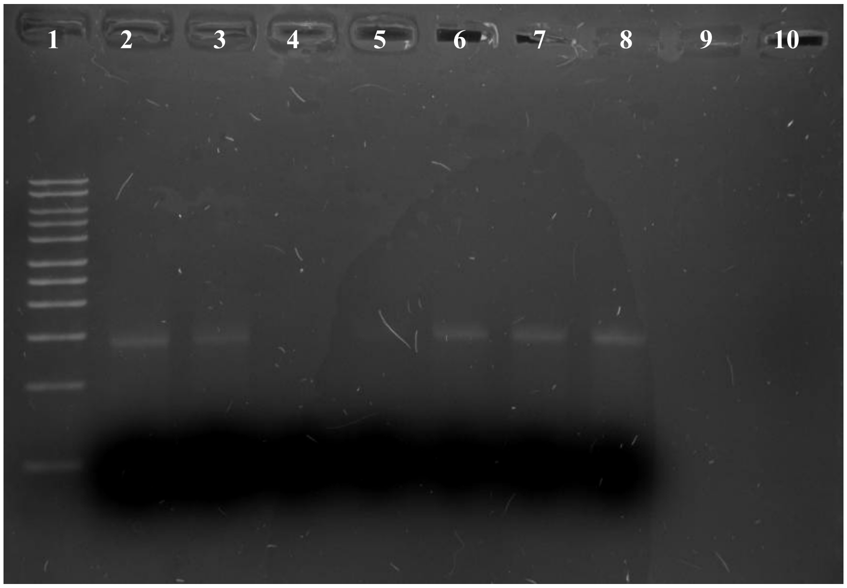
Lane 1- 10k bp DNA ladder
Lane 2- sample 299, core 1 section 1
Lane 3- sample 300, core 1 section 1
Lane 4- sample 301, core 8 section 1
Lane 5- sample 302, core 8 section 1
Lane 6- sample 303, blank
Lane 1- 10k bp ladder (bottom row)
Lane 2- sample 304, core 8 section 2
Lane 3- sample 305, core 8 section 2
Lane 4- sample 306, core 8 section 3
Lane 5- sample 307, core 8 section 3
Lane 6- control

Fig 18k
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 5ul of DNA. Taken 16 Nov, 05. Note sample correspond to slide I.D. 37 on table 6



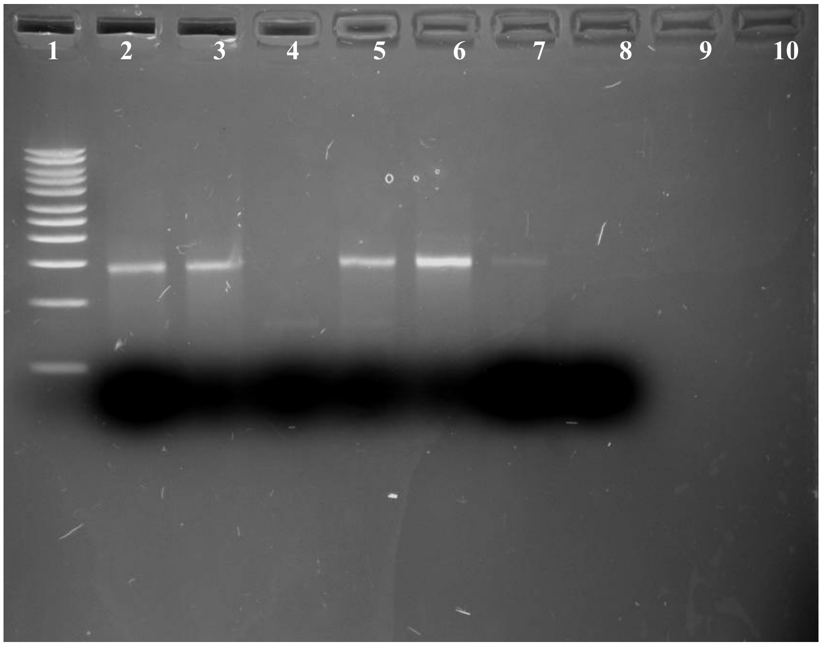
Lane 1- 10k bp DNA ladder
Lane 2- sample 308, core 9 section 1
Lane 3- sample 309, core 9 section 1
Lane 4- sample 310, blank
Lane 5- sample 311, core 9 section 2
Lane 6- sample 312, core 9 section 2
Lane 7- sample 313, core 9 section 3
Lane 8- sample 314, core 9 section 3
Lane 14- control

Fig 18j
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 3ul of DNA. Taken 17 Nov, 05. Note sample correspond to slide I.D. 25 on table 6



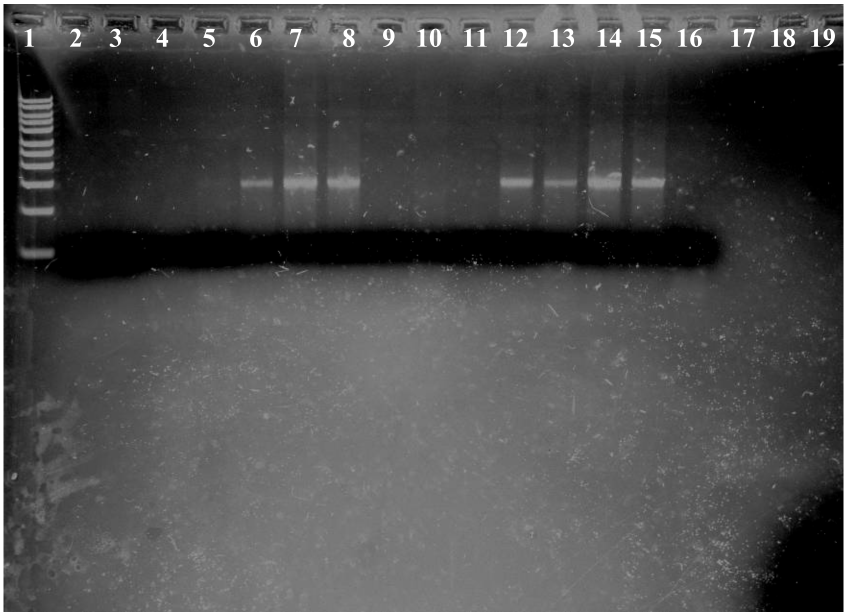
Lane 1- 10k bp III DNA ladder
Lane 2- sample 315, core 8 section 1
Lane 3- sample 316, core 8 section 1
Lane 4- sample 317 blank
Lane 5- sample 318, core 8 section 2
Lane 6- sample 319, core 8 section 2
Lane 7- sample 320, core 8 section 3
Lane 8- sample 321, core 8 section 3
Lane 9- control

Fig 18k
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 3ul of DNA. Taken 17 Nov, 05. Note sample correspond to slide I.D. 26 on table 6



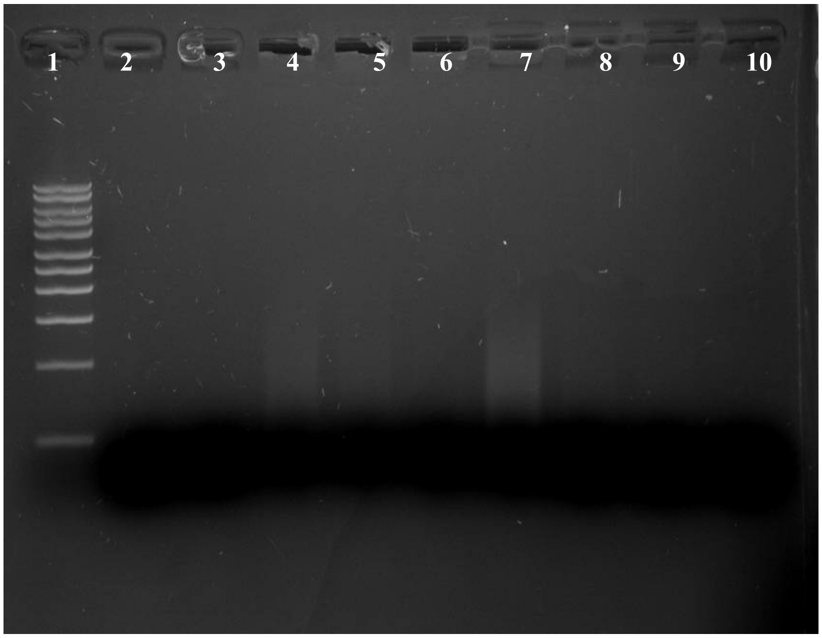
Lane 1- 10k bp DNA ladder
Lane 2- sample 322, core 1 section 2
Lane 3- sample 323, core 7 section 1
Lane 4- sample 324 blank
Lane 5- sample 325, core 1 section 3
Lane 6- sample 326, core 7 section 1
Lane 7- sample 327, core 8 section 3
Lane 8- control

Fig 18l
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 3ul of DNA. Taken 18 Nov, 05. Note sample correspond to slide I.D. 27 on table 6



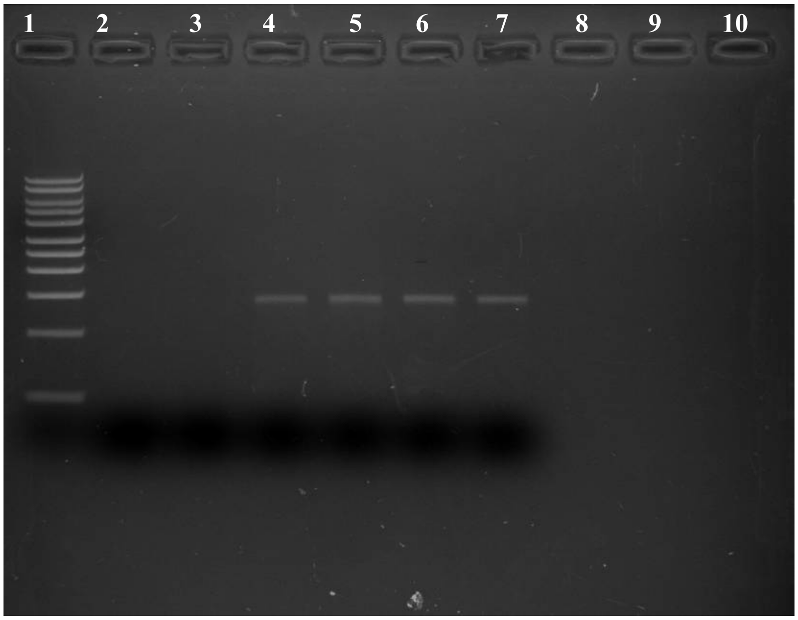
Lane 1- 1k bp DNA ladder
Lane 2- sample 328, core 9 section 3
Lane 3- sample 329, core 9 section 3
Lane 4- sample 330, core 9 section 1
Lane 5- sample 331, core 9 section 1
Lane 6- sample 332, core 6 section 2
Lane 7- sample 333, core 9 section 2
Lane 8- sample 334, core 6 section 2
Lane 9- sample 335, core 9 section 3
Lane 10-sample 336, core 9 section 1
Lane 11- sample 337, core 9 section 1
Lane 12- sample 338, core 9 section 2
Lane 13- sample 339, core 6 section 3
Lane 14- sample 340, core 9 section 2
Lane 15- sample 341, core 9 section 3
Lane 16- sample 342, core 8 section 3
Lane 19- control

Fig 18m
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 2, 3, 5, and 10.5ul of DNA. Taken 18 Nov, 05. Note sample correspond to slide I.D. 28 on table 6



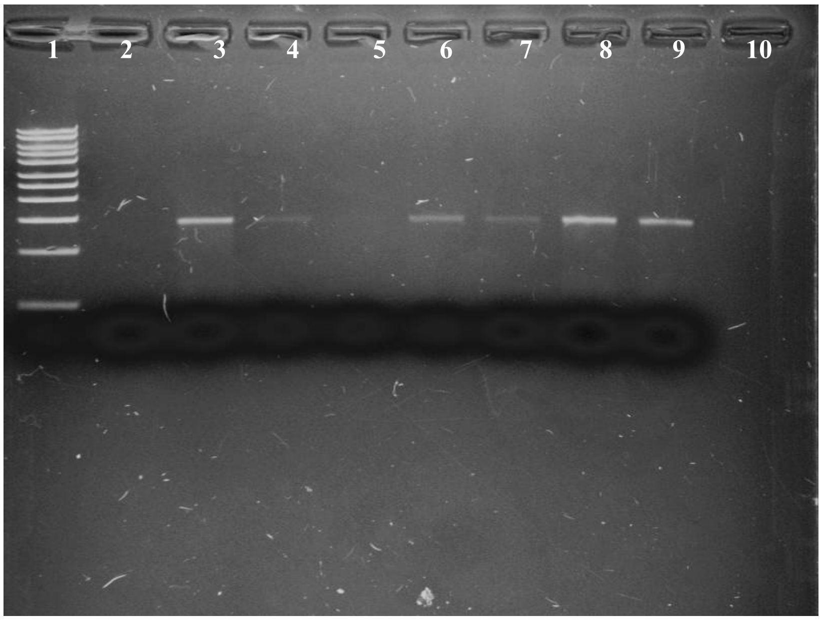
Lane 1- 10k bp DNA ladder
Lane 2- sample 343, core 8 section 1
Lane 3- sample 344, core 8 section 1
Lane 4- sample 345, core 8 section 1
Lane 5- sample 346, core 6 section 3
Lane 6- sample 347, core 9 section 1
Lane 7- sample 348, core 8 section 1
Lane 8- sample 349, core 1 section 2
Lane 9- sample 350, core 9 section 2
Lane 10- sample 351, control

Fig 18n
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 2.5ul of DNA. Taken 16 Dec, 05. Note sample correspond to slide I.D. 29 on table 6



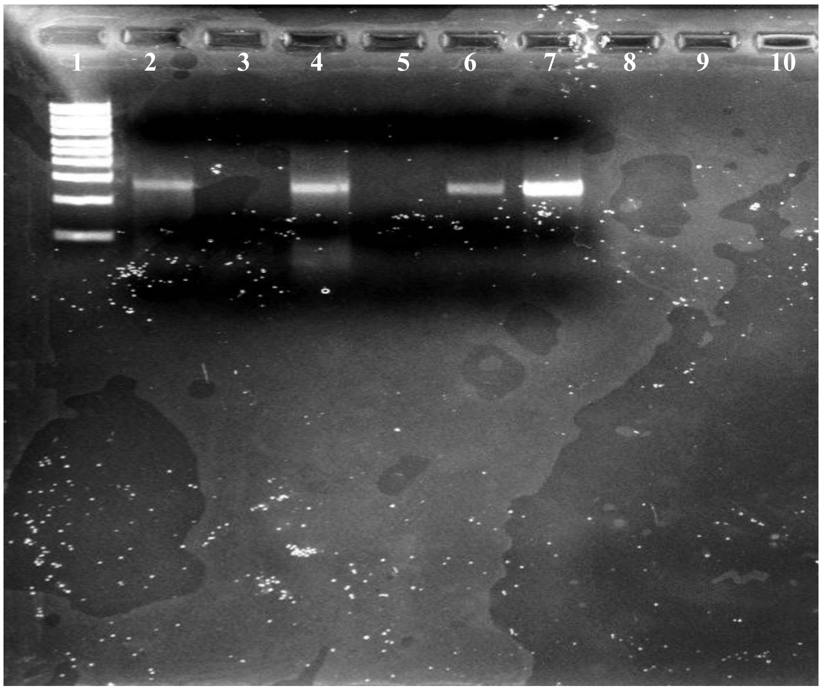
Lane 1- 10k bp DNA ladder
Lane 2- blank
Lane 3- blank
Lane 4- sample 352, core 2 section 1
Lane 5- sample 353, core 2 section 1
Lane 6- sample 354, core 2 section 3
Lane 7- sample 355, core 2 section 3
Lane 8- control

Fig 18o
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 2.5ul of DNA. Taken 16 Dec, 05. Note sample correspond to slide I.D. 38 on table 6



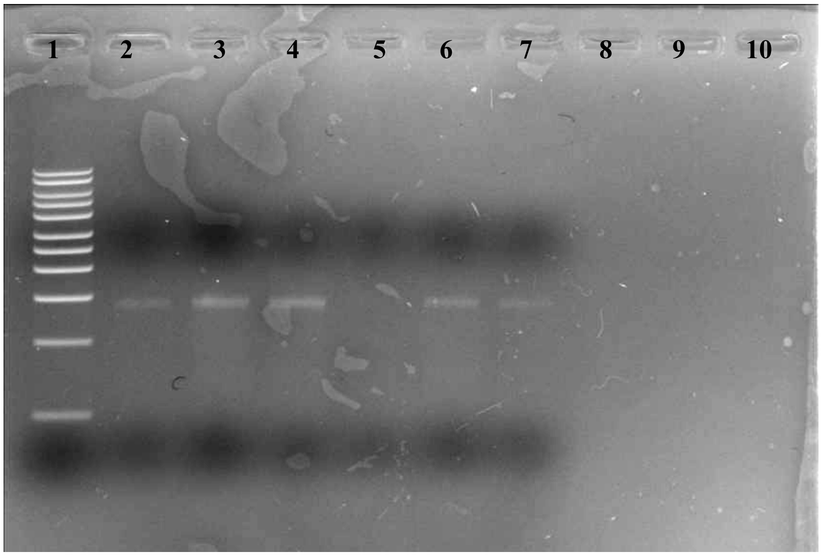
Lane 1- 10k bp DNA ladder
Lane 2- sample 356, core 4 section 1
Lane 3- sample 356, core 4 section 3
Lane 4- sample 357, core 4 section 3
Lane 5- sample blank
Lane 6- sample 358, core 4 section 3
Lane 7- sample 359, core 6 section 2
Lane 8- sample 360, core 6 section 2
Lane 9- sample 361, core 8 section 2
Lane 10- control

Fig 18o
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 3ul of DNA. Taken 29 Dec, 05. Note sample correspond to slide I.D. 30 on table 6



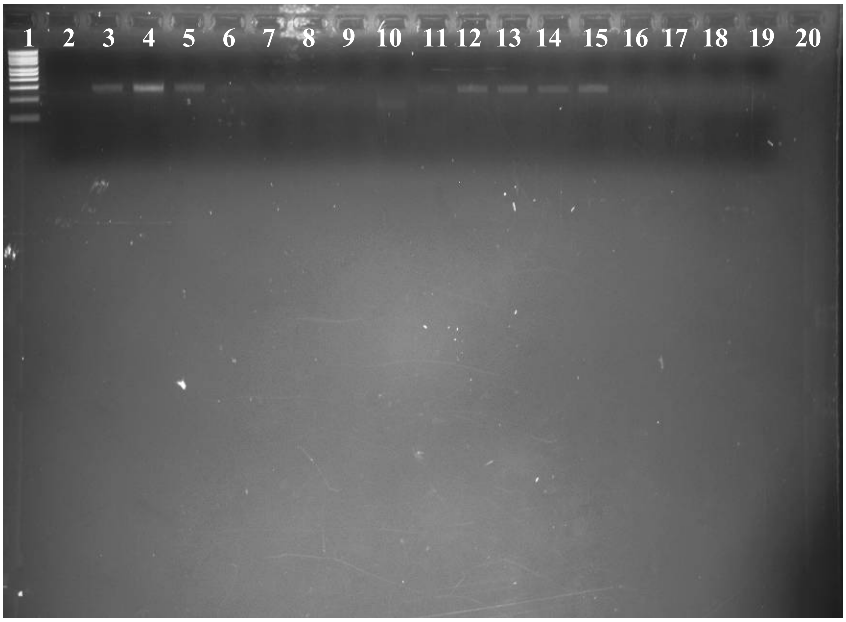
Lane 1- 10k bp DNA ladder
Lane 2- sample 362, core 7 section 1
Lane 3- sample 363, core 7 section 1
Lane 4- sample 364, core 7 section 2
Lane 5- sample 365, core 7 section 2
Lane 6- sample 366, core 8 section 3
Lane 7- sample 367, core 8 section 3
Lane 8- control

Fig 18p
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 3ul of DNA. Taken 3 Jan, 06. Note sample correspond to slide I.D. 31 on table 6



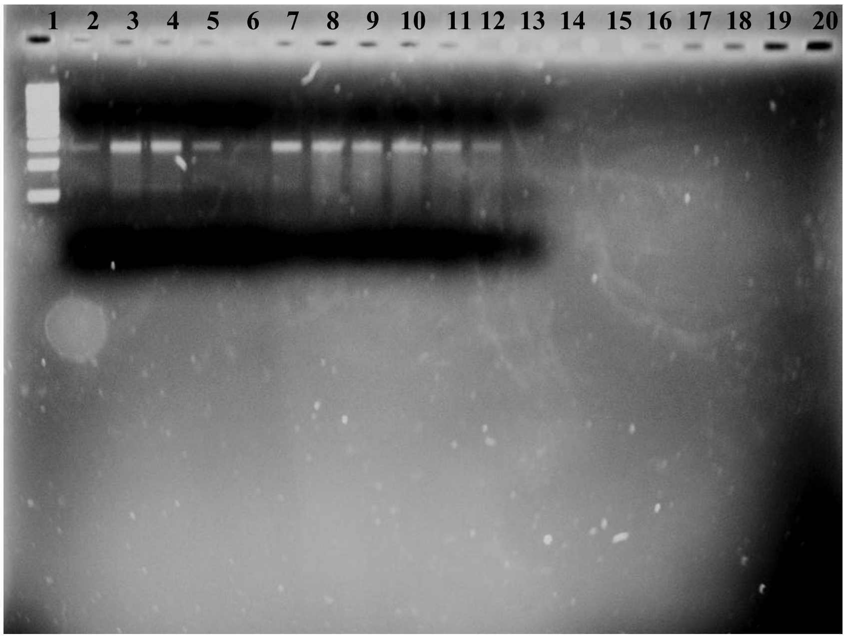
Lane 1- 10k bp DNA ladder
Lane 2- sample 368, core 7 section 3
Lane 3- sample 369, core 7 section 3
Lane 4- sample 370, core 4 section 3
Lane 5- sample 371, core 8 section 3
Lane 6- sample 372, core 8 section 3
Lane 7- sample 373, core 8 section 3
Lane 8- control

Fig 18q
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 3 and 5ul of DNA. Taken 11 Jan, 06. Note sample correspond to slide I.D. 32 on table 6



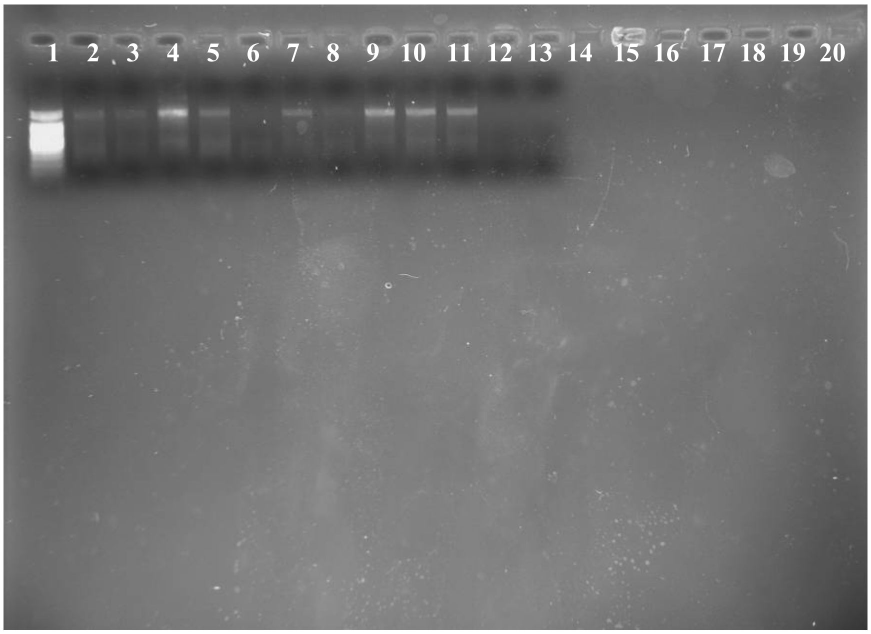
Lane 1- 10k bp DNA ladder
Lane 2- sample 374, core 2 section 1
Lane 3- sample 375, core 2 section 1
Lane 4- sample 376, core 2 section 1
Lane 5- sample 377, core 2 section 2
Lane 6- sample 378, core 2 section 2
Lane 7- sample 379, core 2 section 3
Lane 8- sample 380, core 2 section 3
Lane 9- sample 381, core 6 section 1
Lane 10-sample 382, core 6 section 2
Lane 11- sample 383, core 6 section 2
Lane 12- sample 384, core 6 section 3
Lane 13- sample 385, core 6 section 3
Lane 14- sample 386, core 6 section 1
Lane 15- sample 387, core 4 section 1
Lane 16- sample 388, core 4 section 2
Lane 17- sample 389, core 4 section 2
Lane 18- sample 390, core 4 section 3
Lane 19- sample 391, core 4 section 3
Lane 20- control

Fig 18r
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 2.5 5ul of DNA. Taken 13 Jan, 06. Note sample correspond to slide I.D. 33 on table 6



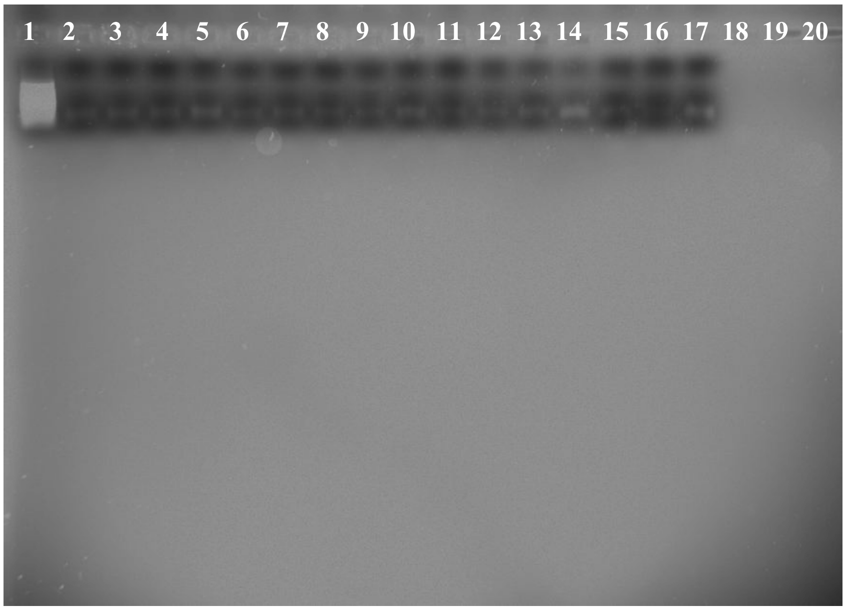
Lane 1- 10k bp DNA ladder
Lane 2- sample 392, core 7 section 1
Lane 3- sample 393, core 7 section 1
Lane 4- sample 394, core 7 section 2
Lane 5- sample 395, core 7 section 2
Lane 6- sample 396, core 7 section 3
Lane 7- sample 397, core 7 section 3
Lane 8- sample 398, core 1 section 1
Lane 9- sample 399, core 1 section 1
Lane 10-sample 400, core 1 section 2
Lane 11- sample 401, core 1 section 2
Lane 12- sample 402, core 1 section 3
Lane 13- sample 403, core 1 section 3
Lane 14- control

Fig 18s
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 2.5 5ul of DNA. Taken 19 Jan, 06. Note sample correspond to slide I.D. 34 on table 6



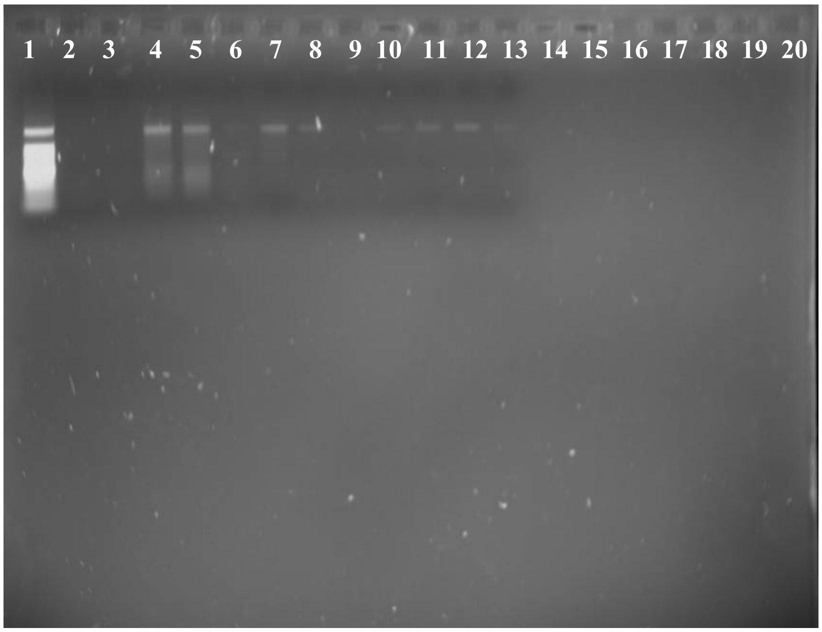
Lane 1- 10k bp DNA ladder
Lane 2- sample 404, core 9 section 1
Lane 3- sample 405, core 9 section 1
Lane 4- sample 406, core 9 section 2
Lane 5- sample 407, core 9 section 2
Lane 6- sample 408, core 9 section 3
Lane 7- sample 409, core 9 section 3
Lane 8- sample 410, core 5 section 1
Lane 9- sample 411, core 5 section 1
Lane 10-sample 412, core 5 section 2
Lane 11- sample 413, core 5 section 2
Lane 12- sample 414, core 5 section 3
Lane 13- sample 415, core 5 section 3
Lane 14- control

Fig 18t
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 2.5 5ul of DNA. Taken 19 Jan, 06. Note sample correspond to slide I.D. 35 on table 6



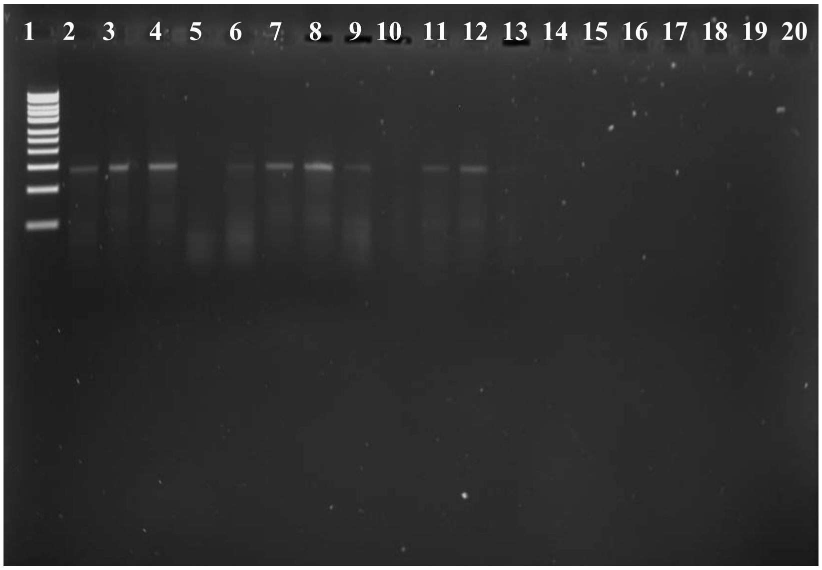
Lane 1- 1k bp DNA ladder
Lane 2- sample 416, core 1 section 1
Lane 3- sample 417, core 1 section 1
Lane 4- sample 418, core 1 section 2
Lane 5- sample 419, core 1 section 2
Lane 6- sample 420, core 1 section 3
Lane 7- sample 421, core 1 section 3
Lane 8- sample 422, core 1 section 3
Lane 9- sample 423, core 1 section 3
Lane 10-sample 424, core 7 section 1
Lane 11- sample 425, core 7 section 1
Lane 12- sample 426, core 7 section 2
Lane 13- sample 427, core 7 section 2
Lane 14- sample 428, core 7 section 3
Lane 15- sample 429, core 7 section 3
Lane 16- sample 430, core 7 section 3
Lane 17- sample 431, core 7 section 3
Lane 18- control

Fig 18u
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 3 and 4ul of DNA. Taken 28 Feb, 06. Note sample correspond to slide I.D. 36 on table 6



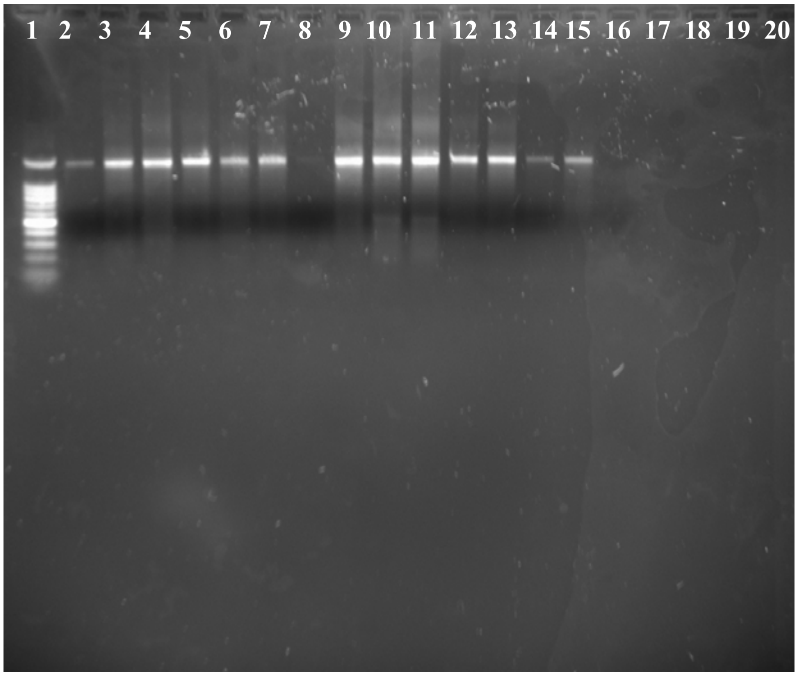
Lane 1- 10k bp DNA ladder
Lane 2- sample 432, core 2 section 1
Lane 3- sample 433, core 2 section 1
Lane 4- sample 434, core 2 section 2
Lane 5- sample 435, core 2 section 2
Lane 6- sample 436, core 2 section 3
Lane 7- sample 437, core 2 section 3
Lane 8- sample 438, core 2 section 1
Lane 9- sample 439, core 2 section 1
Lane 10-sample 440, core 2 section 2
Lane 11- sample 441, core 2 section 2
Lane 12- sample 442, core 2 section 3
Lane 13- sample 443, core 2 section 3
Lane 14- control

Fig 18v
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 2.5ul of DNA. Taken 10 Mar, 06. Note sample correspond to slide I.D. 39 on table 6



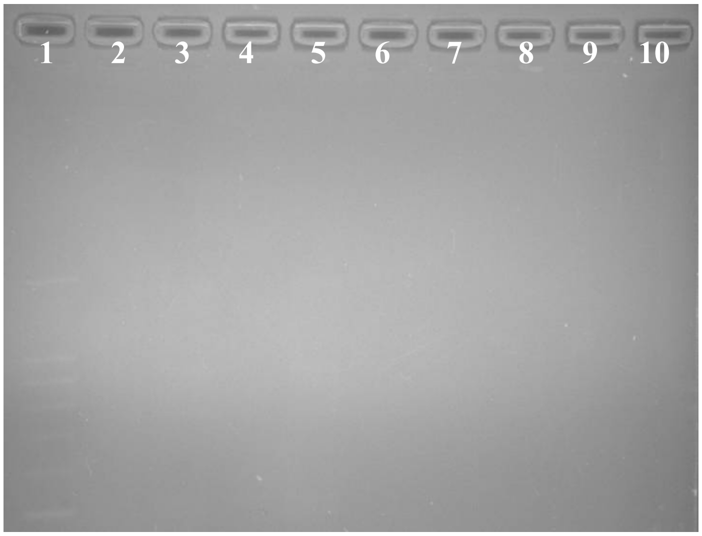
Lane 1- 10k bp DNA ladder
Lane 2- sample 444, core 2 section 2
Lane 3- sample 445, core 2 section 2
Lane 4- sample 446, core 5 section 2
Lane 5- sample 447, core 5 section 2
Lane 6- sample 448, core 7 section 2
Lane 7- sample 449, core 2 section 2
Lane 8- sample 450, core 2 section 2
Lane 9- sample 451, core 7 section 2
Lane 10-sample 452, core 7 section 2
Lane 11- sample 453, core 5 section 2
Lane 12- sample 454, core 5 section 2
Lane 13- sample 455, core 7 section 2
Lane 14- control

Fig 18w
PCR products using universal primers (F)E8f, and (F)AU1406r directly extracted from soil collected from core 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 1% agarose gel for 1hr with 2.5ul of DNA. Taken 11 Sep, 05. Note sample correspond to slide I.D. 12 on table 6



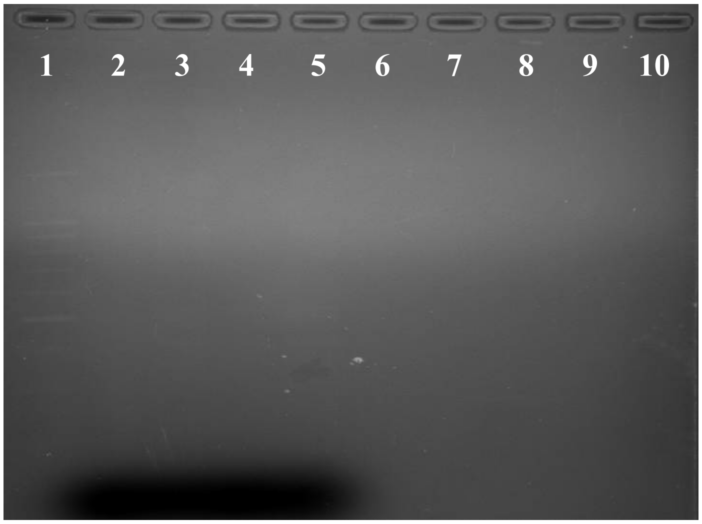
Lane 1- 1k bp DNA ladder
Lane 2- sample 125, core 2 section 1
Lane 3- sample 126, core 2 section 1
Lane 4- sample 127, core 2 section 2
Lane 5- sample 128, core 2 section 2
Lane 6- sample 129, core 2 section 3
Lane 7- sample 130, core 2 section 3
Lane 8- sample 131, core 5 section 1
Lane 9- sample 132, core 5 section 1
Lane 10-sample 133, core 5 section 1
Lane 11- sample 134, core 5 section 1
Lane 12- sample 135, core 5 section 2
Lane 13- sample 136, core 5 section 2
Lane 14- sample 137, core 5 section 3
Lane 15- sample 138, core 5 section 3
Lane 16- control

Fig 19a
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 5, and 6 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2% agarose gel for 2 hr and restricted with Rsa1 with 18ul of DNA. Taken 8 Dec, 05. Note sample corresponds to slide #1 on table 8



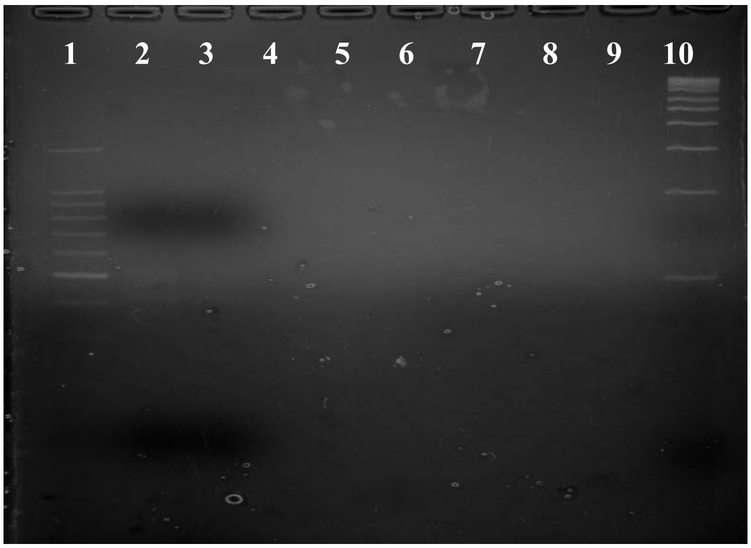
Lane 1- 100 bp DNA ladder
Lane 2- sample 138, core 5 section 3
Lane 3- sample 195, core 5 section 2
Lane 4- sample 88, core 6 section 3
Lane 5- control

Fig 19b
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 2 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2% agarose gel for 2 hr and restricted with Rsa1 with 18ul of DNA. Taken 12 Dec, 05. Note sample corresponds to slide #2 on table 8



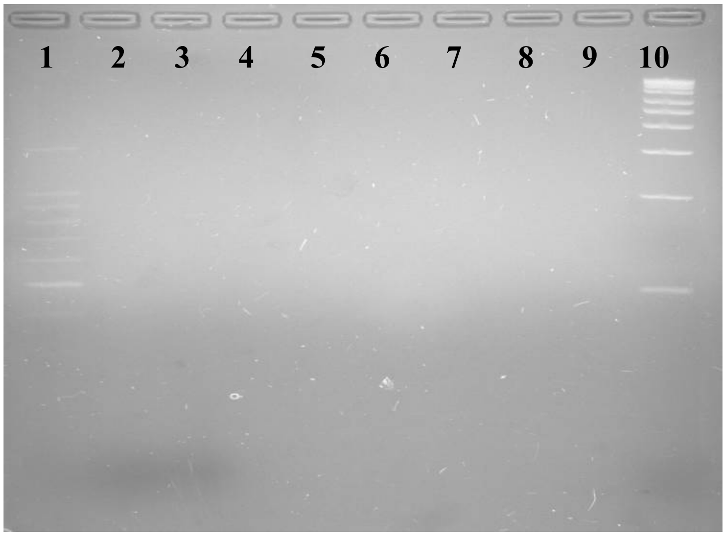
Lane 1- 100 bp DNA ladder
Lane 2- sample 129, core 2 section 3
Lane 3- sample 185, core 2 section 1
Lane 4- control

Fig 19c
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 8 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2% agarose gel for 2 hr and restricted with Rsa1 with 18ul of DNA. Taken 15 Dec, 05. Note sample corresponds to slide #3 on table 8



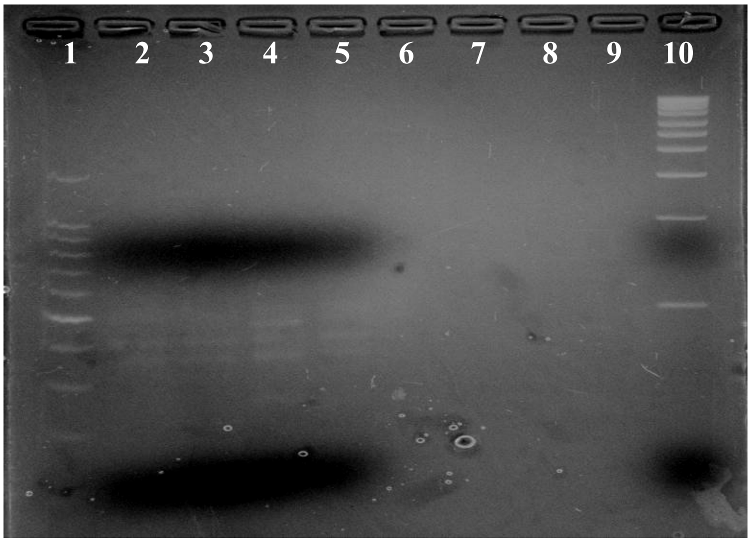
Lane 1- 100 bp DNA ladder
Lane 2- sample 316, core 8 section 1
Lane 3- sample 327, core 8 section 3
Lane 4- control
Lane 10 10K bp DNA ladder

Fig 19d
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 6 and 8 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2% agarose gel for 2 hr and restricted with Rsa1 with 18ul of DNA. Taken 16 Dec, 05. Note sample corresponds to slide #4 on table 8



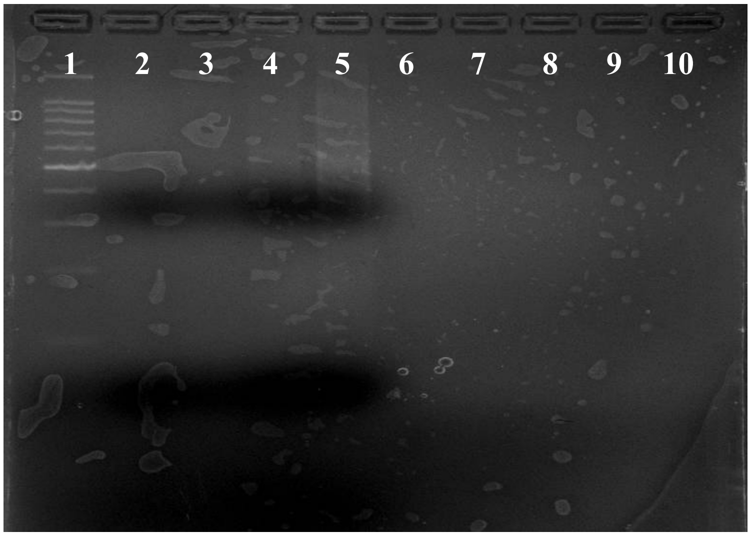
Lane 1- 100 bp DNA ladder
Lane 2- sample 360, core 6 Section 2
Lane 3- sample 361, core 8 section 2
Lane 4- control
Lane 10- 10k bp DNA ladder

Fig 19e
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 2, 6 and 8 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2% agarose gel for 2 hr and restricted with Rsa1 with 18ul of DNA. Taken 18 Dec, 05. Core 6, section 2 has bands @ 550, 400, and 200 bp; Core 8 section 2 has bands @ 550, 400, and 200 bp; Core 2, section 2 has bands @ 550, 400, 350, and 250; Core 8, section 3 has bands @ 550, 400, and 350. Note sample corresponds to slide #5 on table 8



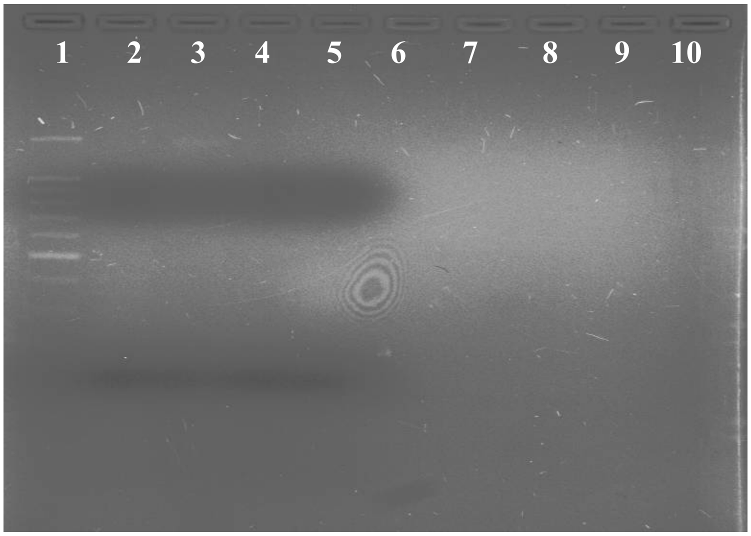
Lane 1- 100 bp DNA ladder
Lane 2- sample 360, core 6 Section 2
Lane 3- sample 361, core 8 section 2
Lane 4- sample 127, core 2 section 2
Lane 5- sample 342, core 8 section 3
Lane 6- control
Lane 10 10k bp DNA ladder

Fig 19f
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 2, and 8 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 3% agarose gel for 2 hr and restricted with Rsa1 with 18ul of DNA. Taken 30 Dec, 05. Note sample corresponds to slide #6 on table 8



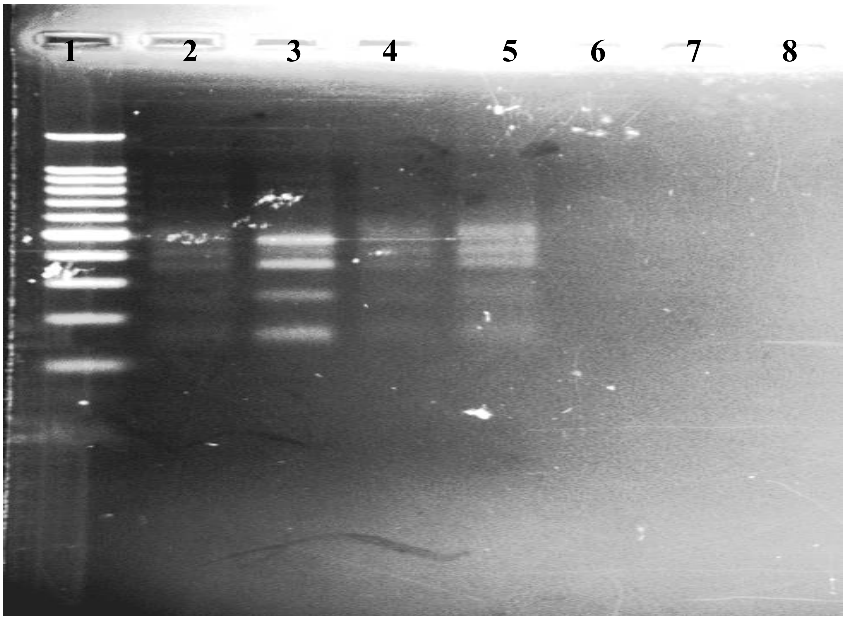
Lane 1- 100 bp DNA ladder
Lane 2- sample 320, core 8 Section 3
Lane 3- sample 321, core 8 section 3
Lane 4- sample 166, core 2 section 3
Lane 5- sample 167, core 2 section 3
Lane 6- control

Fig 19g
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 8 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 3% agarose gel for 2 hr and restricted with Rsa1 with 18ul of DNA. Taken 4 Jan, 06. Note sample corresponds to slide #7 on table 8



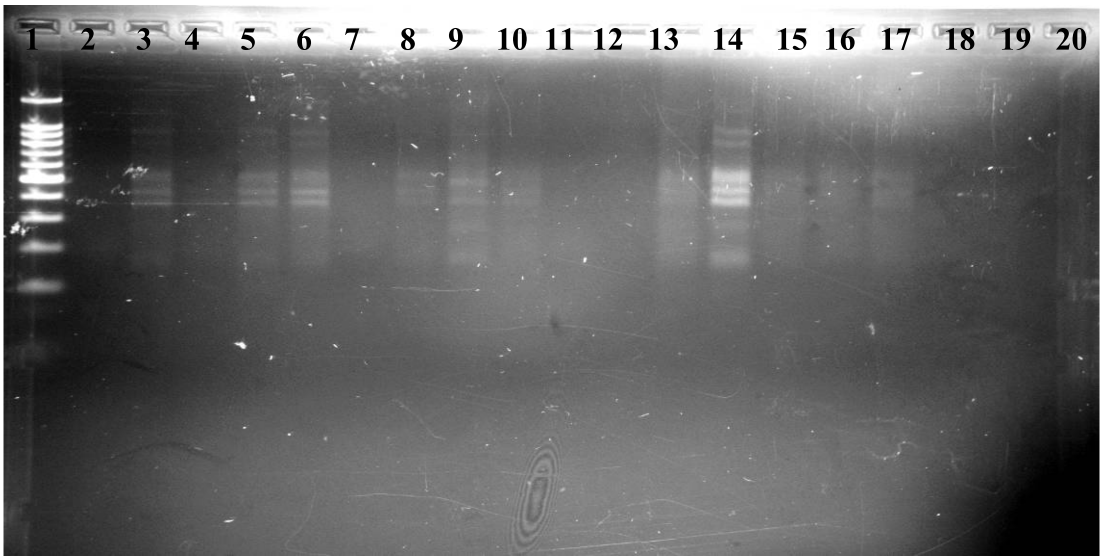
Lane 1- 100 bp DNA ladder
Lane 2- sample 320, core 8 Section 3
Lane 3- sample 321, core 8 section 3
Lane 4- sample 372, core 8 section 3
Lane 5- sample 373, core 8 section 3
Lane 6- control

Fig 19h
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 1,2, and 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2% agarose gel for 2 hr and restricted with Rsa1 with 18ul of DNA. Taken 5 Jan, 06. Core 2, section 2 has bands @ 550, 400, 350, 250 and 150 bp. Note sample corresponds to slide #8 on table 8



Lane 1- 100 bp DNA ladder
Lane 2- sample 127, core 2 Section 2
Lane 3- sample 128, core 2 section 2
Lane 4- sample 187, core 2 section 2
Lane 5- sample 188, core 2 section 2
Lane 6- control

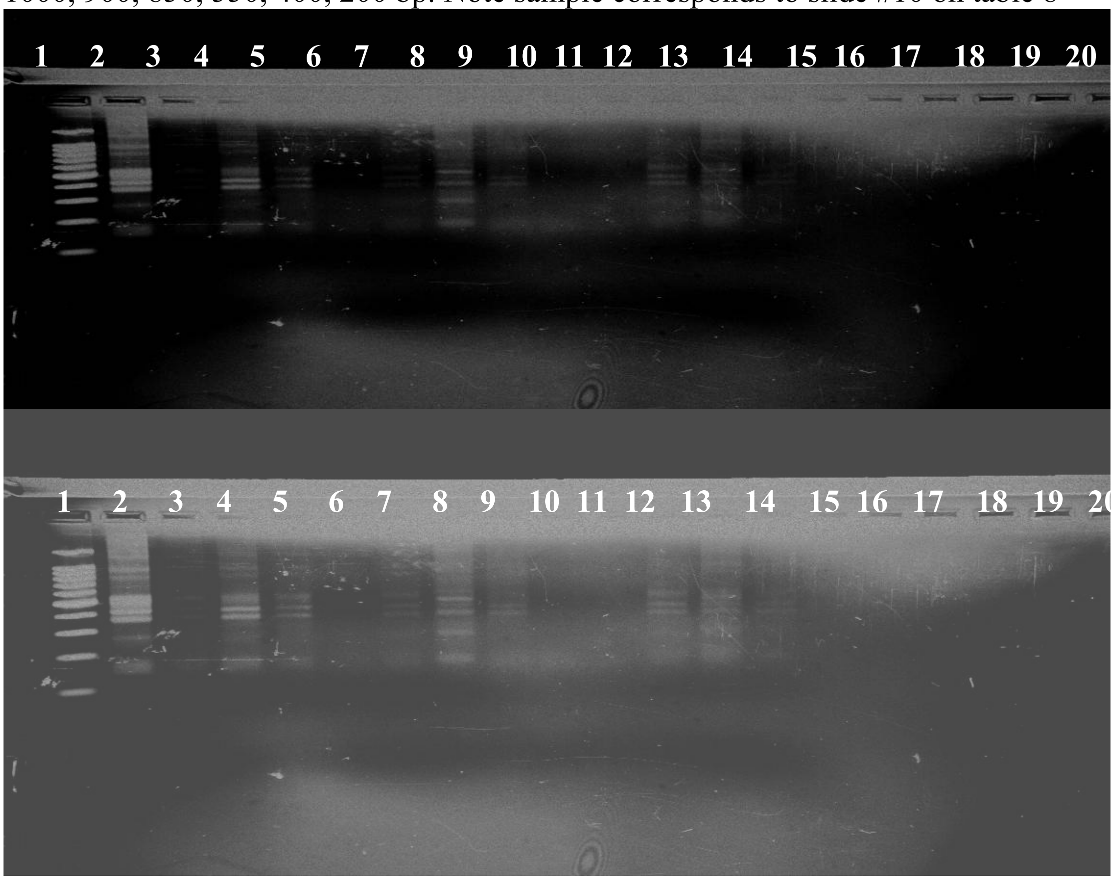
Fig 19i
1 PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 1, 2, and 4 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2% agarose gel for 2 hr and restricted with Rsa1 with 17.5ul of DNA. Taken 6 Jan, 06. Same samples will be run at 2.5 agarose to increase separation of bands for 2.5 hr. Core 1, section 1 has bands @ 550, 400, and 200 bp; section 2 has bands @ 1200, 900, 850, 550, 400, 300, and 165 section 3 has bands @ 900, 850, 550, 400, 300, and 150 bp. Core 2 section 2 has bands @ 550, 400, 400, 200, and bp; section 3 has bands @ 550, 400, 400, 200 bp; Core 4 section 1 has bands @ 1000, 900, 850, 550, 400, 400, 200 bp; section 2 has bands @ 900, 850, 550, 400, and 200 bp. Note sample corresponds to slide #9 on table 8



Lane 1- 100 bp DNA ladder
Lane 2- sample 299, core 1 section 1
Lane 3- sample 300, core 1 section 2
Lane 4- sample 222, core 1 section 1
Lane 5- sample 325, core 1 section 3
Lane 6- sample 322, core 1 section 3
Lane 7- sample 349, core 1 section 2
Lane 8- sample 352, core 2 section 2
Lane 9- sample 353, core 2 section 2
Lane 10-sample 354, core2 section 3
Lane 11-sample 355, core 2 section 3
Lane 12- sample 356, core 4 section 1
Lane 13- sample 364, core 4 section 1
Lane 14- sample 45, core 4 section 2
Lane 15-sample 56, core 4 section 2
Lane 16-sample 357 core 4 section 3
Lane 17-sample 370 core 4 section 3
Lane 18-control

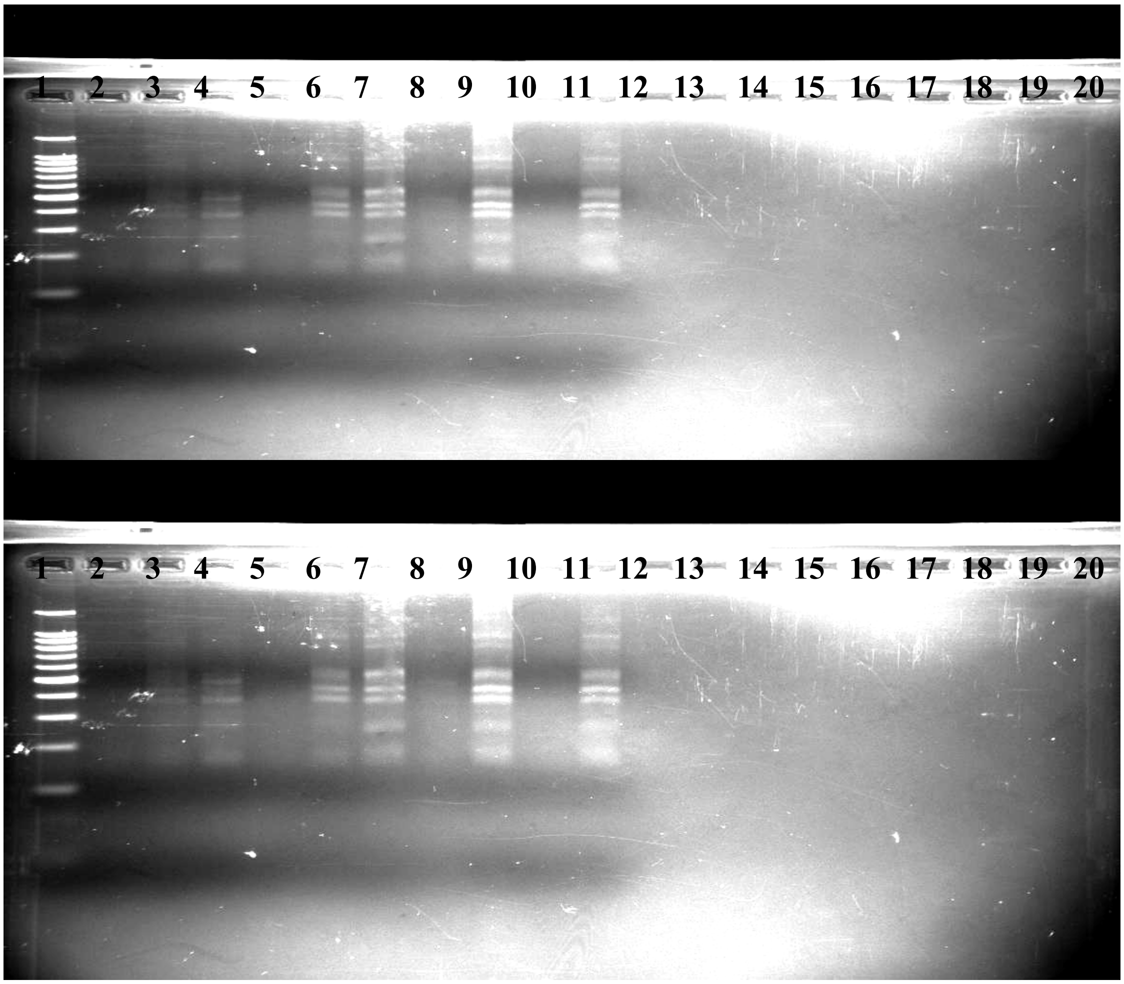
Fig 19j

PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 1,2, 4, and 5 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2.5% agarose gel for 2.5 hr and digested with Rsa1 and 17.5 ul of DNA. Taken on 8 Jan, 06. Core 5, section 1 has bands @ 1300, 900, 800, 550, 500, 250, 200, and 150 bp; core 1 section 1 has bands @ 1300, 900, 800, 550, 500, 300, and 200, and 150; core 1 section 3 cannot be determined. Core 2 section 1 has bands @ 600, 550, 400, 200, and 150 bp; section 3 cannot be determined. Core 4 section 1 has bands @ 900, 800, 550, 400, 200 bp; section 3 has bands @ 1000, 900, 850, 550, 400, 200 bp. Note sample corresponds to slide #10 on table 8



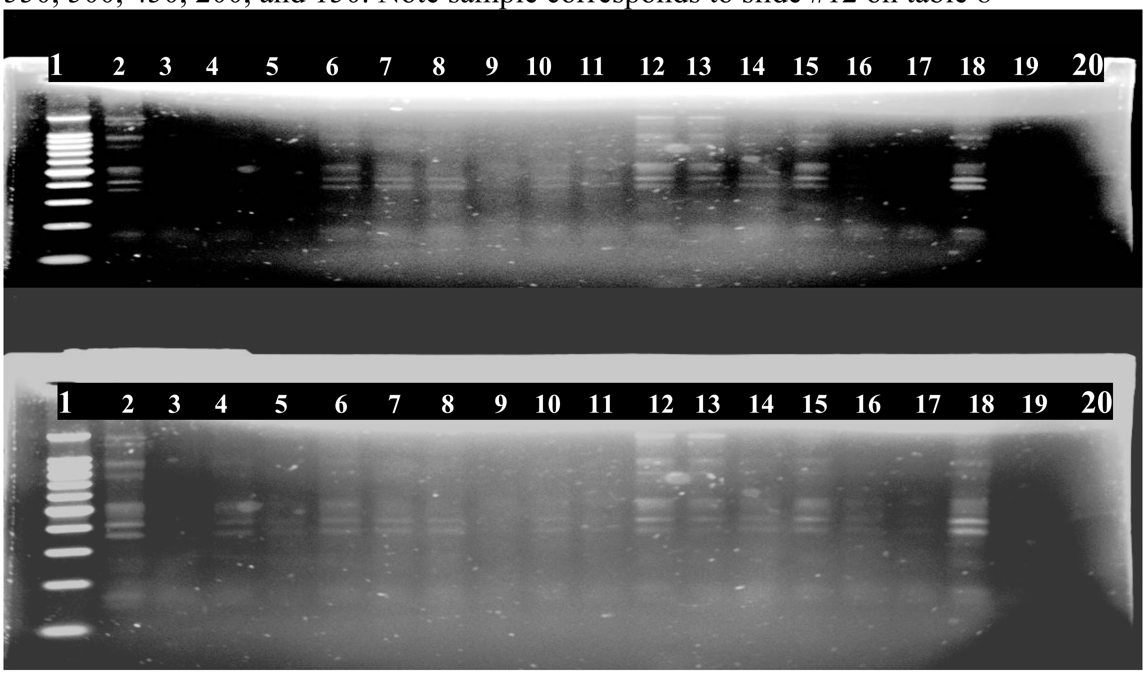
Lane 1- 100 bp DNA ladder
Lane 2- sample 131, core 5 section 1
Lane 3- sample 132, core 5 section 1
Lane 4- sample 322, core 1 section 3
Lane 5- sample 325, core 1 section 3
Lane 6- sample 349, core 1 section 2
Lane 7- sample 352, core 2 section 2
Lane 8- sample 353, core 2 section 2
Lane 9- sample 354, core 2 section 3
Lane 10-sample 355, core 4 section 3
Lane 11- sample 356, core 4 section 1
Lane 12- sample 357, core 4 section 3
Lane 13- sample 364, core 4 section 1
Lane 14-sample 370, core 4 section 3
Lane 15-control

Fig 19k
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 5 and 6 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2.5% agarose gel. For 2.5 hr and digested with Rsa1 with 10.5 ul of DNA. Taken 9 Jan, 06. Core 5, section 2 cannot be determined section 3 has bands @ 500, 450, 300, and 150 bp; core 6 section 1 has bands @ 900, 800, 550, 500, 250, and 200; section 2 cannot be determined; section 3 has bands @ 900, 800, 550, 500, 300, and 200bp. Note sample corresponds to slide #11 on table 8



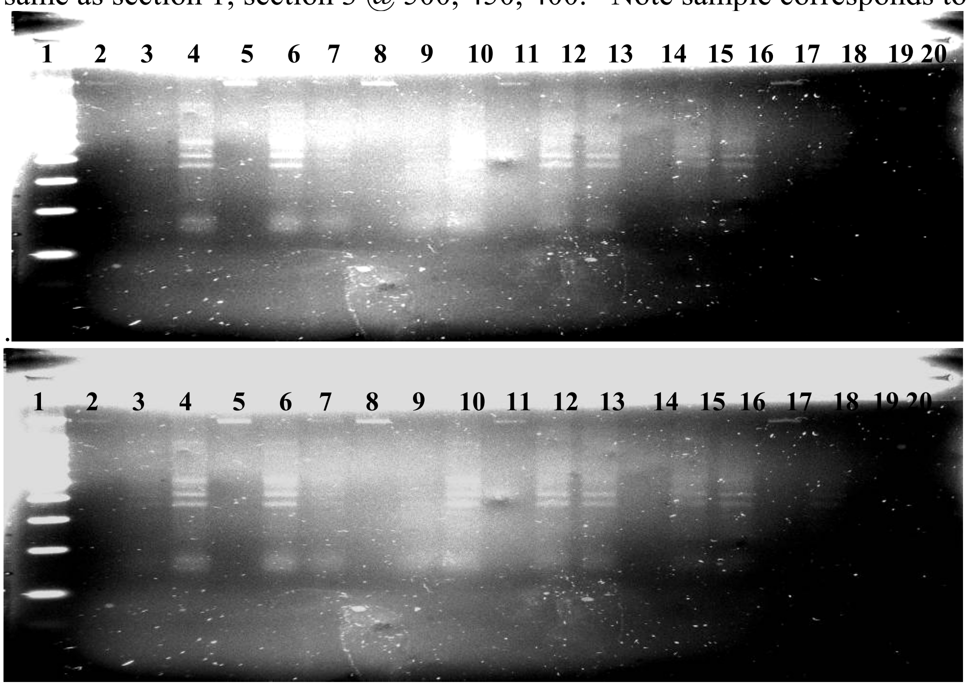
Lane 1- 100 bp DNA ladder
Lane 2- sample 195, core 5 section 2
Lane 3- sample 69, core 5 section 3
Lane 4- sample 137, core 5 section 3
Lane 5- sample 196, core 5 section 2
Lane 6- sample 211, core 6 section 1
Lane 7- sample 212, core 6 section 1
Lane 8- sample 332, core 6 section 2
Lane 9- sample 346, core 6 section 3
Lane 10-sample 334, core 6 section 2
Lane 11- sample 339, core 6 section 3
Lane 12-control

Fig 19l
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 6, 7, 8, and 9 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2.5% agarose gel for 3.5 hr and restricted with Rsa1 with 14ul of DNA. Taken 13 Jan, 06. Note other figures are the same but recorded at different resolutions for better visualization of bands. Core 7, section 1 has bands @ 1400, 1300, 900, 800, 500, 450, 400, 200, and 150 bp; section 2 exhibits the same as section 1 except for the; section 3 the same as section1. Core 8 section 1 bands @ 1300, 900, 800, 500, 450, 400, 250, 200, bp; section 2 has bands @ 500, 450, 400, 200, and 150 bp, section 3 has bands the same as section 2; Core 9 section 3 has bands @ 1400, 1000, 900, 850, 550, 500, 450, 200, and 150 bp; core 6 section 3 has bands @ 900, 850, 550, 500, 450, 200, and 150. Note sample corresponds to slide #12 on table 8



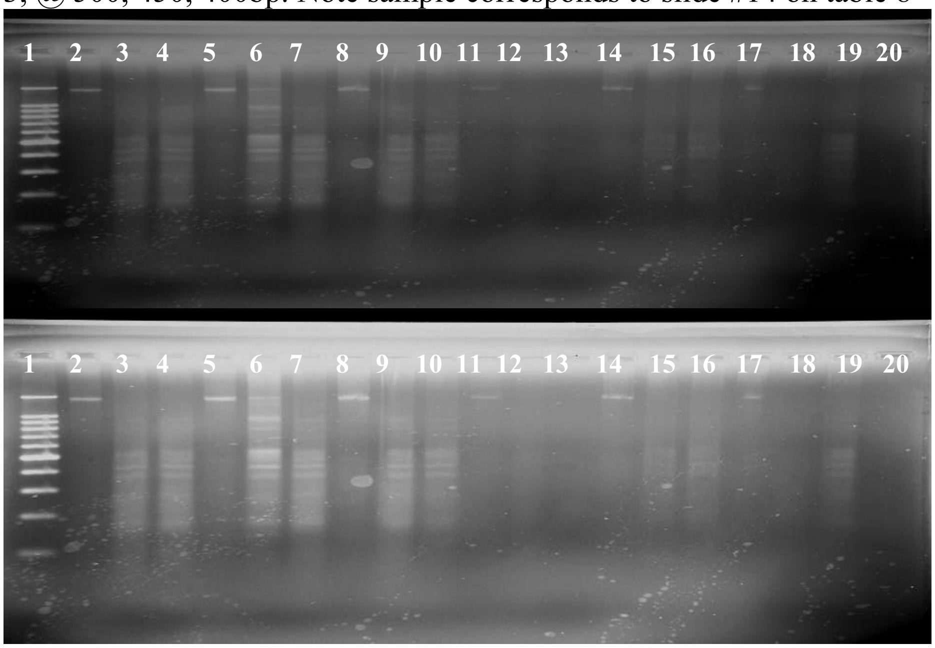
Lane 1- 100 bp DNA ladder
Lane 2- sample 326, core 7 section 1
Lane 3- sample 362, core 7 section 1
Lane 4- sample 80, core 7 section 2
Lane 5- sample 368, core 7 section 3
Lane 6- sample 369, core 7 section 3
Lane 7- sample 315, core 8 section 1
Lane 8- sample 316, core 8 section 1
Lane 9- sample 318, core 8 section 2
Lane 10-sample 319, core 8 section 2
Lane 11- sample 327, core 8 section 3
Lane 12- sample 341, core 9 section 3
Lane 13- sample 335, core 9 section 3
Lane 14- sample 75, core 6 section 3
Lane 15- sample 339, core 6 section 3
Lane 16- sample 372, core 8 section 3
Lane 17- sample 373, core 8 section 3
Lane 18 – sample 323, core 7 section 2
Lane 19- sample 332, core 6 section 1
Lane 20- control

Fig 19m
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 7 and 1 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2.5% agarose gel for 3.5 hr and restricted with Rsa1 for 2 hr with 12, 10, and 14ul of DNA. Taken 18 Jan, 06. Note other figures are the same but recorded at different resolutions for better visualization of bands. Core 7 section 1 has bands @ 900, 500, 450, 400, 200, and 150 bp; section 2 and 3 same as section 1. Core 1, section 1 has bands @ 500, 450, 400, 200; section 2 same as section 1; section 3 @ 500, 450, 400. Note sample corresponds to slide #13 on table 8



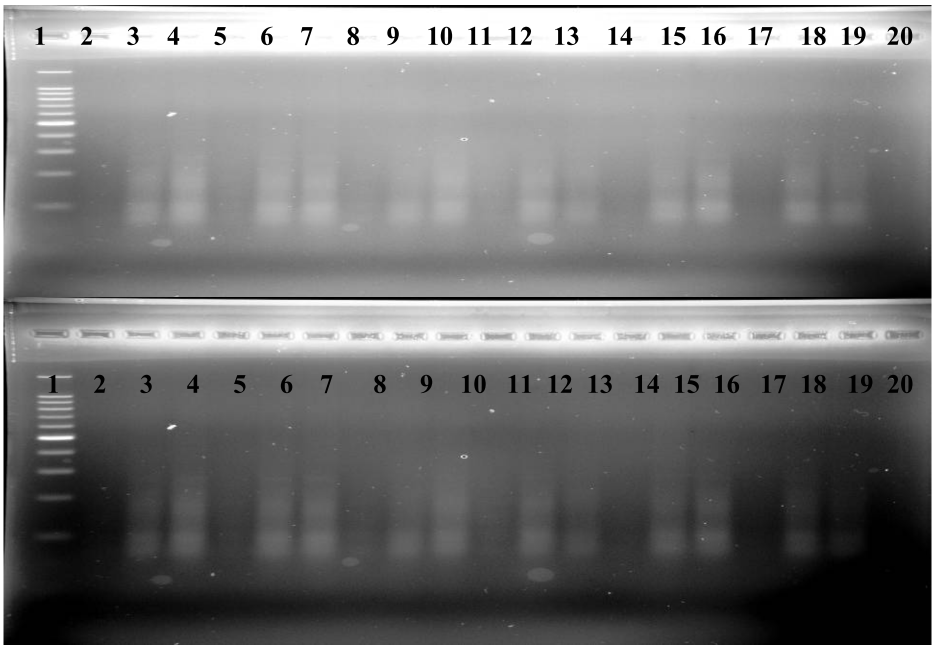
Lane 1- 100 bp DNA ladder
Lane 2- sample 392, core 7 section 1, not digested
Lane 3- sample 392, core 7 section 1
Lane 4- sample 393, core 7 section 1
Lane 5- sample 394, core 7 section 2, not digested
Lane 6- sample 394, core 7 section 2
Lane 7- sample 395, core 7 section 2
Lane 8- sample 396, core 7 section 3, not digested
Lane 9- sample 396, core 7 section 3
Lane 10-sample 397, core 7 section 3
Lane 11- sample 398, core 1 section1, not digested
Lane 12- sample 398, core 1 section 1
Lane 13- sample 399, core 1 section 1
Lane 14- sample 400, core 1section 2, not digested
Lane 15- sample 400, core 1 section 2
Lane 16- sample 401, core 1 section 2
Lane 17- sample 402, core 1 section 3, not digested
Lane 18 – sample 402, core 1 section 3
Lane 19- sample 403, core 1 section 3
Lane 20- control

Fig 19n
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 9 and 5 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2.5% agarose gel for 3.5 hr and restricted with Rsa1 for 2 hr with 14ul of DNA. Taken 19 Jan, 06. Note other figures are the same but recorded at different resolutions for better visualization of bands. Core 9 section 1 has bands @ 900, 800, 500, 450, 400, 300, and 250 bp; section 2 has bands @ 1400 (undigested), 900, 800, 500, 450, 400, 300, 250, section 3 exhibits the same as section 2. Core 5, section 1 cannot be determined; section 2, 500 450, and 400 bp; section 3, @ 500, 450, 400bp. Note sample corresponds to slide #14 on table 8



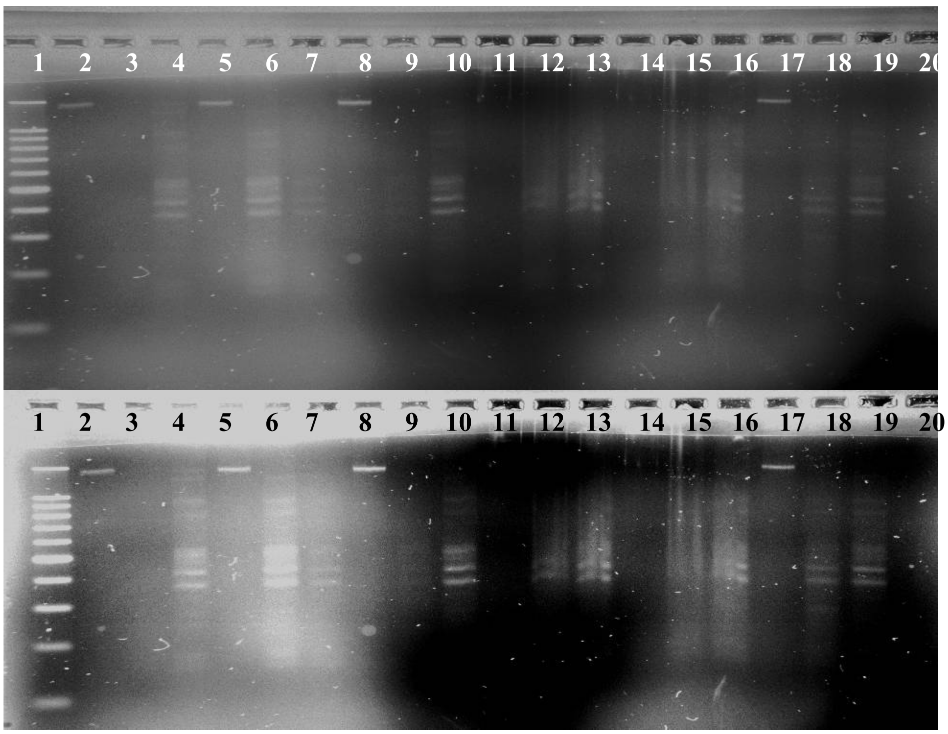
Lane 1- 100 bp DNA ladder
Lane 2- sample 404, core 9 section 1, not digested
Lane 3- sample 404, core 9 section 1
Lane 4- sample 405, core 9 section 1
Lane 5- sample 406, core 9 section 2, not digested
Lane 6- sample 406, core 9 section 2
Lane 7- sample 407, core 9 section 2
Lane 8- sample 408, core 9 section 3, not digested
Lane 9- sample 408, core 9 section 3
Lane 10-sample 409, core 9 section 3
Lane 11- sample 410, core 5 section1, not digested
Lane 12- sample 410, core 5 section 1
Lane 13- sample 411, core 5 section 1
Lane 14- sample 412 core 5 section 2, not digested
Lane 15- sample 412, core 5 section 2
Lane 16- sample 413, core 5 section 2
Lane 17- sample 415, core 5 section 3, not digested
Lane 18 – sample 414, core 5 section 3
Lane 19- sample 415, core 5 section 3
Lane 20- control

Fig 19o
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 1 and 7 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2.5% agarose gel for 3.5 hr and restricted with Rsa1for 2 hr @ 14ul of DNA. Taken 20 Jan, 06. Note other figures are the same but recorded at different resolutions for better visualization of bands. This sample must be re-taken. Don't know why results inaccurate. Perhaps running gel contaminated thus inhibiting results because lanes 2, 5, 8, 11, 14, and 17 were positive non-digested DNA samples but now appear as negative. Note sample corresponds to slide #15 on table 8



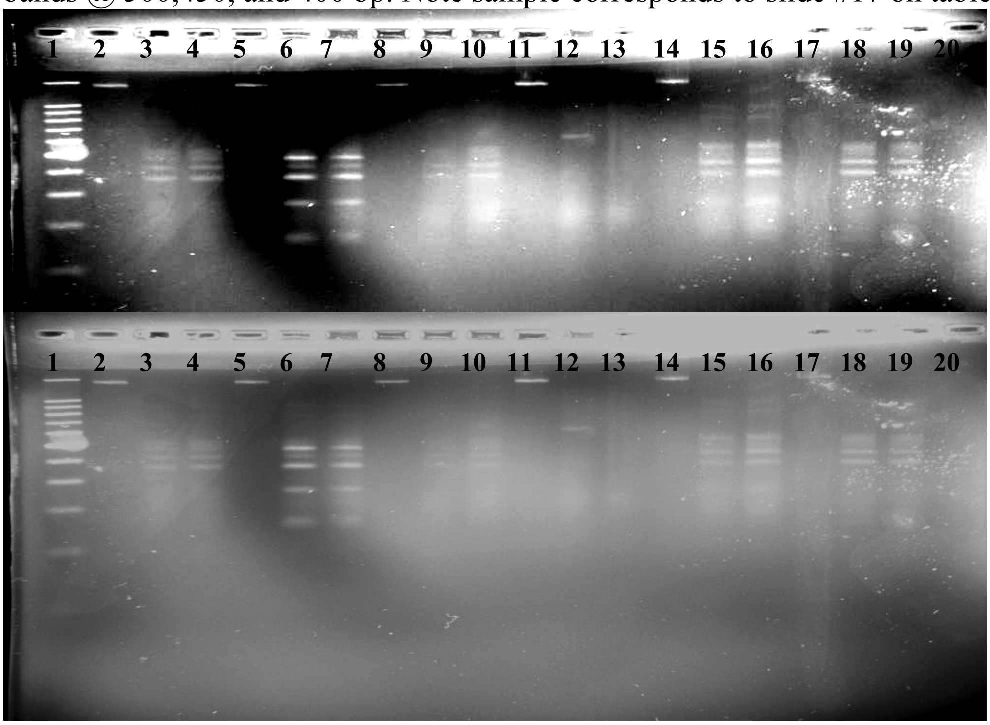
Lane 1- 100 bp DNA ladder
Lane 2- sample 416, core 1 section 1, not digested
Lane 3- sample 417, core 1 section 1
Lane 4- sample 418, core 1 section 1
Lane 5- sample 419, core 1 section 2, not digested
Lane 6- sample 420, core 1 section 2
Lane 7- sample 421, core 1 section 2
Lane 8- sample 422, core 1 section 3, not digested
Lane 9- sample 423, core 1 section 3
Lane 10-sample 424, core 1 section 3
Lane 11- sample 425, core 7 section1, not digested
Lane 12- sample 426, core 7 section 1
Lane 13- sample 427, core 7 section 1
Lane 14- sample 428, core 7 section 2, not digested
Lane 15- sample 429, core 7 section 2
Lane 16- sample 430, core 7 section 2
Lane 17- sample 431, core 7 section 3, not digested
Lane 18 – sample 432, core 7 section 3
Lane 19- sample 433, core 7 section 3
Lane 20- control

Fig 19p
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 7 and 1 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2.5% agarose gel for 3.5 hr and restricted with Rsa1 for 2 hr with 12, 10, and 14ul of DNA. Taken 23 Jan, 06. Note other figures are the same but recorded at different resolutions for better visualization of bands. Core 7 section 1 has bands @ 1400, 1300, 900, 800, 550, 450, 400, 200, and 150 bp; section 2 has bands @ 1300, 900, 800, 550, 500, 450, 200, and 150; section 3 same as section 2. Core 1, section 1 has bands @ 500, 450 and 400; section 2 has bands @ 900, 800, 500, 450, 400; section 3 has bands @ 900, 800, 500, 450, 400bp. Note sample corresponds to slide #16 on table 8



Lane 1- 100 bp DNA ladder
Lane 2- sample 392, core 7 section 1, not digested
Lane 3- sample 392, core 7 section 1
Lane 4- sample 393, core 7 section 1
Lane 5- sample 394, core 7 section 2, not digested
Lane 6- sample 394, core 7 section 2
Lane 7- sample 395, core 7 section 2
Lane 8- sample 396, core 7 section 3, not digested
Lane 9- sample 396, core 7 section 3
Lane 10-sample 397, core 7 section 3
Lane 11- sample 398, core 1 section1, not digested
Lane 12- sample 398, core 1 section 1
Lane 13- sample 399, core 1 section 1
Lane 14- sample 400, core 1section 2, not digested
Lane 15- sample 400, core 1 section 2
Lane 16- sample 401, core 1 section 2
Lane 17- sample 402, core 1 section 3, not digested
Lane 18 – sample 402, core 1 section 3
Lane 19- sample 403, core 1 section 3
Lane 20- control

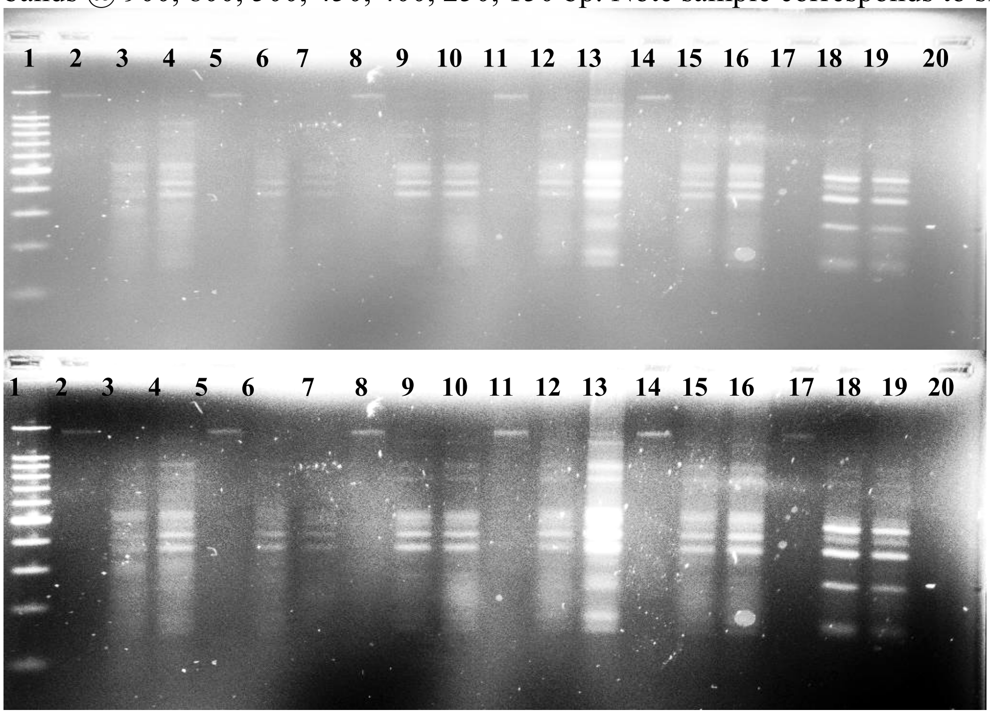
Fig 19q
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 2 and 6 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2.5% agarose gel for 3.5 hr and restricted with Rsa1 for 2 hr with 12 and 14ul of DNA. Taken 23 Jan, 06. Note other figures are the same but recorded at different resolutions for better visualization of bands. Core 2, section 1 has bands @ 900, 800, 500, 450, 400bp; section 2 has bands @ 900, 800, 500, 450, 400, 250, and 150; section 3 has bands @ 500, 450, 400. Core 6, section 1 @ 500,450, 400bp; section 2 has bands @900, 800, 550, 500, 450, and 400; section 3 has bands @ 500,450, and 400 bp. Note sample corresponds to slide #17 on table 8



Lane 1- 100 bp DNA ladder
Lane 2- sample 375, core 2 section 1, not digested
Lane 3- sample 375, core 2 section 1
Lane 4- sample 376, core 2 section 1
Lane 5- sample 377, core 2 section 2, not digested
Lane 6- sample 377, core 2 section 2
Lane 7- sample 378, core 2 section 2
Lane 8- sample 379, core 2 section 3, not digested
Lane 9- sample 379, core 2 section 3
Lane 10-sample 380, core 2 section 3
Lane 11- sample 381, core 6 section 2, not digested
Lane 12- sample 381, core 6 section 2
Lane 13- sample 382, core 6 section 2
Lane 14- sample 383, core 6 section 3, not digested
Lane 15- sample 383, core 6 section 3
Lane 16- sample 384, core 6 section 3
Lane 17- sample 385, core 6 section 1, not digested
Lane 18 – sample 385, core 6 section 1
Lane 19- sample 386, core 6 section 1
Lane 20- control

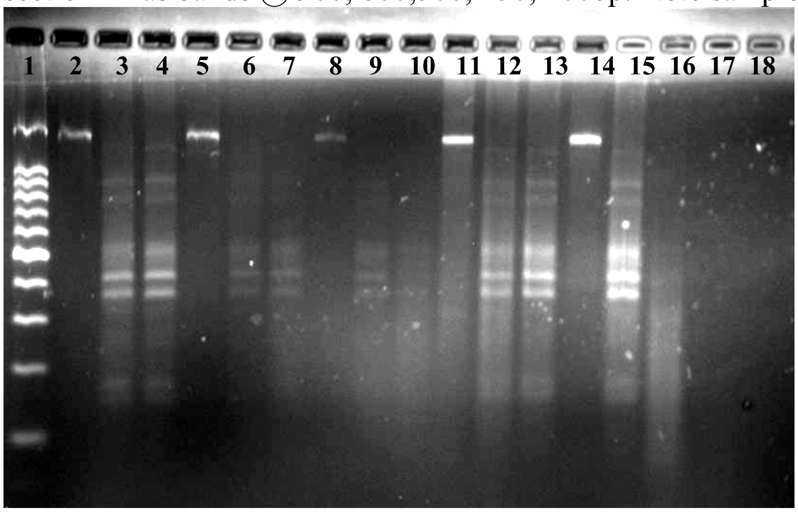
Fig 19r

PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 4, 5, 7, and 8 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2.5% agarose gel for 3.5 hr and restricted with Rsa1 for 2 hr with 12 and 14ul of DNA. Taken 27 Jan, 06. Note other figures are the same but recorded at different resolutions for better visualization of bands. Core 4, section 1 has bands @ 900, 800, 500, 450, 400, 200, and 150 bp; section 2 has bands @ 900, 800, 500, 450, 400; section 3 has bands @ 1300, 900, 800, 500, 450, 400, 250, 200 and 150. Core 5, section 1 has bands @ 1300, 900, 800, 500, 450, 400, 250, 200, and 150; Core 7 section 1 has bands @ 1300, 900, 800, 500, 450, 400; Core 8 section 3 has bands @ 900, 800, 500, 450, 400, 250, 150 bp. Note sample corresponds to slide #18 on table 8



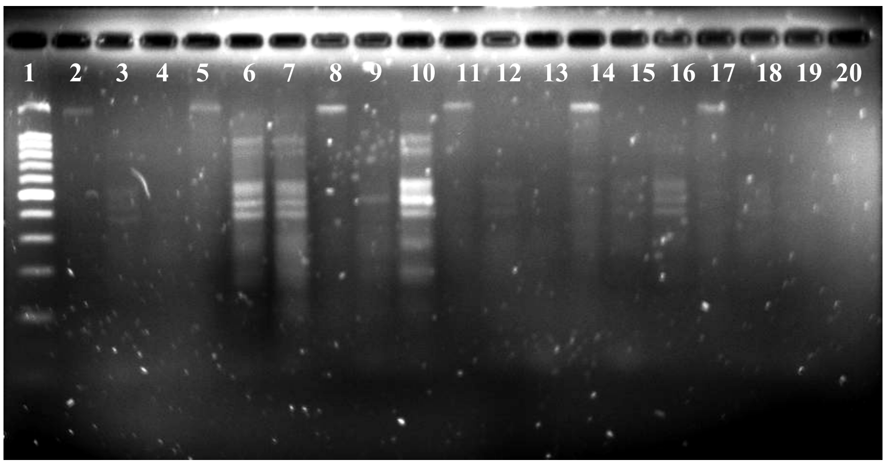
Lane 1- 100 bp DNA ladder
Lane 2- sample 356, core 4 section 1, not digested
Lane 3- sample 356, core 4 section 1
Lane 4- sample 388, core 4 section 1
Lane 5- sample 45, core 4 section 2, not digested
Lane 6- sample 45, core 4 section 2
Lane 7- sample 56, core 4 section 2
Lane 8- sample 357, core 4 section 3, not digested
Lane 9- sample 357, core 4 section 3
Lane 10-sample 370, core 4 section 3
Lane 11- sample 410, core 5 section 1, not digested
Lane 12- sample 410, core 5 section 1
Lane 13- sample 411, core 5 section 1
Lane 14- sample 392, core 7 section 1, not digested
Lane 15- sample 392, core 7 section 1
Lane 16- sample 393, core 7 section 1
Lane 17- sample 372, core 8 section 3, not digested
Lane 18 – sample 372, core 8 section 3
Lane 19- sample 373, core 8 section 3
Lane 20- control

Fig 19s
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 2 and 7 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2.5% agarose gel for 3.5 hr and restricted with Rsa1 for 2 hr with 12 and 14ul of DNA. Taken 14 Mar, 06. Core 2, section 2 has bands @ 900, 800, 500, 450, 400, 200 and 150bp; Core 7, section 2 has bands @ 900, 800,500, 450, 400bp. Note sample corresponds to slide #18 on table 8



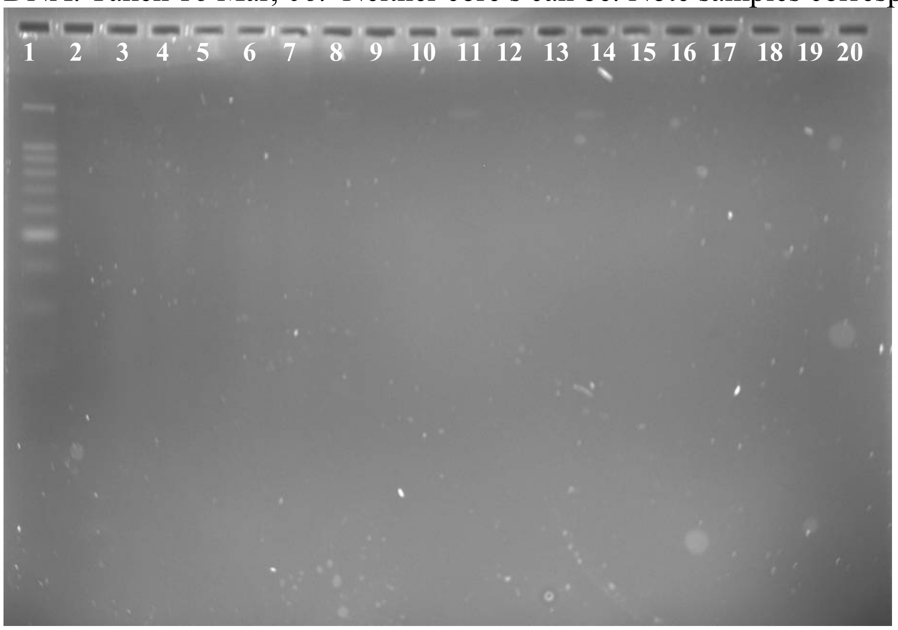
Lane 1- 100 bp DNA ladder
Lane 2- sample 434, core 2 section 2, not digested
Lane 3- sample 434, core 2 section 2
Lane 4- sample 435, core 2 section 2
Lane 5- sample 448, core 7 section 2, not digested
Lane 6- sample 448, core 7 section 2
Lane 7- sample 451, core 7 section 2
Lane 8- sample 452, core 7 section 2, not digested
Lane 9- sample 452, core 7 section 2
Lane 10-sample 455, core 7 section 2
Lane 11- sample 440, core 2 section 2, not digested
Lane 12- sample 440, core 2 section 2
Lane 13- sample 441, core 2 section 2
Lane 14- sample 442, core 2 section 2, not digested
Lane 15- sample 442, core 2 section 2
Lane 16- sample 443, core 2 section 2
Lane 17- control

Fig 19t
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 2, 5, 6, 7, 8, and 9 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2.5% agarose gel for 3.5 hr and restricted with Rsa1 for 2 hr with 12 and 14ul of DNA. Taken 15 Mar, 06. Core 9, section 2 has bands @ 550, 450, and 400bp; Core 5, section 2 has bands @ 900, 800, 500, 450, 400, 250, 200bp; Core 8 section 1 same as core 5 section 2 but higher intensity and 1300 bp; Core 2, section 3 has bands @ 500, 450, and 400bp; Core 7 section 1 has bands @ 900, 800, 500, 450, and 400bp; Core 6 section 2 500,450, 400. Note samples corresponds to slide #19 on table 8



Lane 1- 100 bp DNA ladder
Lane 2- sample 406, core 9 section 2, not digested
Lane 3- sample 406, core 9 section 2
Lane 4- sample 407, core 9 section 2
Lane 5- sample 446, core 5 section 2, not digested
Lane 6- sample 446, core 5 section 2
Lane 7- sample 447, core 5 section 2
Lane 8- sample 315, core 8 section 1, not digested
Lane 9- sample 315, core 8 section 1
Lane 10-sample 316, core 8 section 1
Lane 11- sample 436, core 2 section 3, not digested
Lane 12- sample 436, core 2 section 3
Lane 13- sample 437, core 2 section 3
Lane 14- sample 392, core 7 section 1, not digested
Lane 15- sample 392, core 7 section 1
Lane 16- sample 393, core 7 section 1
Lane 17- sample 372, core 6 section 2, not digested
Lane 18 – sample 372, core 6 section 2
Lane 19- sample 373, core 6 section 2
Lane 20- control

Fig 19u
PCR products using universal primers (F)E8f and (F)AU1406r directly extracted from soil collected from cores 6, 8, and 9 wetland. PCR product of interest is 1390 bp in length. Samples were run on a 2.5% agarose gel for 3.5 hr and restricted with Rsa1 for 2 hr with 12 and 14ul of DNA. Taken 16 Mar, 06. Neither core s can be. Note samples corresponds to slide #21 on table 8



Lane 1- 100 bp DNA ladder
Lane 2- sample 384, core 6 section 3, not digested
Lane 3- sample 384, core 6 section 3
Lane 4- sample 385, core 6 section 3
Lane 5- sample 372, core 8 section 3, not digested
Lane 6- sample 372, core 8 section 3
Lane 7- sample 373, core 8 section 3
Lane 8- sample 408, core 9 section 3, not digested
Lane 9- sample 408, core 9 section 3
Lane 10-sample 409, core 9 section 3
Lane 11- control

Table 3: Core I.D.

Core ID	Date Taken	Season	Area Taken	Section	Notes
1	15-Jan-05	Winter	Valle Greene	N/A	00 VALLE GREEN WINTER
2	13-Feb-05	Winter	WPAFB	N/A	01 WPAFB WINTER
3	2-May-05	Spring	WPAFB	N/A	USED FOR GREENHOUSE
4	2-May-05	Spring	WPAFB	N/A	05020502 WPAFB SPRING
5	2-May-05	Spring	Valle Greene	N/A	05020503 VALLE GREENE SPRING
6	20-Jul-05	Summer	WPAFB	N/A	05020505 WPAFB SUMMER
7	22-Jul-05	Summer	Valle Greene	N/A	05020504 VALLE GREENE SUMMER
8	20-Oct-05	Fall	WPAFB	N/A	N/A
9	20-Oct-05	Fall	Valle Greene	N/A	N/A

Table 4: Tube I.D.

Core V Tube ID#	Core ID	Section	A/B	Date	Note and Core Information (if applicable)
1	6	1	A	28-Jun-05	05020505A Summer WPAFB
2	6	1	B	28-Jun-05	05020505A Summer WPAFB
3	6	2	A	28-Jun-05	05020505A Summer WPAFB
4	6	2	B	28-Jun-05	05020505A Summer WPAFB
5	6	3	A	28-Jun-05	05020505A Summer WPAFB
6	6	3	B	28-Jun-05	05020505A Summer WPAFB
7	4	1	A	28-Jun-05	05020502 SPRING WPAFB
8	4	1	B	28-Jun-05	05020502 SPRING WPAFB
9	4	2	A	28-Jun-05	05020502 SPRING WPAFB
10	4	2	B	28-Jun-05	05020502 SPRING WPAFB
11	4	3	A	28-Jun-05	05020502 SPRING WPAFB
12	4	3	B	28-Jun-05	05020502 SPRING WPAFB
13	4	X	A	28-Jun-05	05020502 SPRING WPAFB BLANK1
14	4	X	B	28-Jun-05	05020502 SPRING WPAFB BLANK2
15	5	1	A	5-Mar-05	05020503 SPRING VALLE GREENE
16	5	1	B	5-Mar-05	05020503 SPRING VALLE GREENE
17	5	2	A	5-Mar-05	05020503 SPRING VALLE GREENE
18	5	2	B	5-Mar-05	05020503 SPRING VALLE GREENE
19	5	3	A	5-Mar-05	05020503 SPRING VALLE GREENE
20	5	3	B	5-Mar-05	05020503 SPRING VALLE GREENE
21	5	X	A	5-Mar-05	05020503 SPRING VALLE GREENE BLANK1
22	5	X	B	5-Mar-05	05020503 SPRING VALLE GREENE BLANK2
23	7	1	A	5-Mar-05	05020504 SUMMER VALLE GREENE
24	7	1	B	5-Mar-05	05020504 SUMMER VALLE GREENE
25	7	2	A	5-Mar-05	05020504 SUMMER VALLE GREENE
26	7	2	B	5-Mar-05	05020504 SUMMER VALLE GREENE
27	7	3	A	5-Mar-05	05020504 SUMMER VALLE GREENE
28	7	3	B	5-Mar-05	05020504 SUMMER VALLE GREENE
29	1	1	A	15-Jan-05	LOG # 1293 WINTER VALLE GREENE
30	1	1	B	15-Jan-05	LOG # 1294 WINTER VALLE GREENE
31	1	2	A	15-Jan-05	LOG # 1295 WINTER VALLE GREENE
32	1	2	B	15-Jan-05	LOG # 1296 WINTER VALLE GREENE
33	1	3	A	15-Jan-05	LOG # 1297 WINTER VALLE GREENE
34	1	3	B	15-Jan-05	LOG # 1298 WINTER VALLE GREENE
35	2	1	A	13-Feb-05	LOG # 1305 WINTER WPAFB
36	2	1	B	13-Feb-05	LOG # 1306 WINTER WPAFB
37	2	2	A	13-Feb-05	LOG # 1310 WINTER WPAFB
38	2	2	B	13-Feb-05	LOG # 1311 WINTER WPAFB
39	2	3	A	13-Feb-05	LOG # 1320 WINTER WPAFB
40	2	3	B	13-Feb-05	LOG # 1321 WINTER WPAFB
41	4	2	A	12-Sep-04	
42	4	2	A	12-Sep-04	
43	4	2	A	12-Sep-04	
44	4	3	A	12-Sep-04	
45	4	3	A	12-Sep-04	

46	4	3	A	12-Sep-04	
47	4	3	A	12-Sep-04	
48	2	X	A	12-Sep-04	
49	2	X	B	12-Sep-04	
50	2	1	A	12-Sep-04	
51	2	1	A	12-Sep-04	
52	2	2	A	12-Sep-04	
53	2	2	A	12-Sep-04	
54	2	3	A	12-Sep-04	
55	2	3	A	12-Sep-04	
56	2	X	A	12-Sep-04	
57	2	X	B	12-Sep-04	
58	2	1	A	12-Sep-04	
59	2	2	A	12-Sep-04	
60	2	3	A	12-Sep-04	
61	1	1	A	15-Jan-05	
62	1	2	A	15-Jan-05	
63	1	3	A	15-Jan-05	
64	5	1	A	5-Mar-05	
65	5	2	A	5-Mar-05	
66	5	3	A	5-Mar-05	
67	6	1	A	28-Jun-05	
68	6	2	A	28-Jun-05	
69	6	3	A	28-Jun-05	
70	7	1	A	5-Mar-05	
71	7	2	A	5-Mar-05	
72	7	3	A	5-Mar-05	
73	5	X	A	5-Mar-05	
74	7	1	A	5-Mar-05	
75	7	1	A	5-Mar-05	
76	7	2	A	5-Mar-05	
77	7	2	A	5-Mar-05	
78	7	1	A	5-Mar-05	
79	8	1	A	15-Jun-05	
80	8	1	A	15-Jun-05	
81	8			15-Jun-05	did not use
82	8	X	A	15-Jun-05	blank
83	8	2	A	15-Jun-05	
84	8	2	A	15-Jun-05	
85	8			15-Jun-05	did not use
86	8	X	A	15-Jun-05	blank
87	8	3	A	15-Jun-05	
88	8	3	A	15-Jun-05	
89				15-Jun-05	did not use
90	9	1	A	15-Jun-05	
91	9	1	A	15-Jun-05	
92	9			15-Jun-05	did not use
93	9	X	A	15-Jun-05	blank

94	9	2	A	15-Jun-05	
95	9	2	A	15-Jun-05	
96	9			15-Jun-05	did not use
97	9	X	A	15-Jun-05	blank
98	9	3	A	15-Jun-05	
99	9	3	A	15-Jun-05	
100				15-Jun-05	did not use
101	X	X		15-Jun-05	water

Table 5: Template I.D.

Template ID#	Core ID	Section	A/B	Date	Note and Core Information (if applicable)	Old ID
1	7	1	A	9-Sep-05	#05020504 Section 1 taken July 05 Valle Green	1A1
2	6	1	A	9-Sep-05	#05020505 Section 1 taken July 05 WPAFB cell#1	1A1
3	5	1	A	8-Jul-05	#05020503 Section 1 taken May 05 Valle Green	1A1503
4	7	1	A	9-Sep-05	#05020504 Section 1 taken July 05 Valle Green	1A2
5	6	1	A	9-Sep-05	#05020505 Section 1 taken July 05 WPAFB cell#1	1A2
6	5	1	A	8-Jul-05	#05020503 Section 1 taken May 05 Valle Green	1A2503
7	5	1	A	8-Jul-05	#05020503 Section 1 taken May 05 Valle Green	1B1503
8	5	1	A	8-Jul-05	#05020503 Section 1 taken May 05 Valle Green	1B2503
9	7	2	A	9-Sep-05	#05020504 Section 2 taken July 05 Valle Green	2A1
10	6	2	A	9-Sep-05	#05020505 Section 2 taken July 05 WPAFB cell#1	2A1
11	5	2	A	8-Jul-05	#05020503 Section 2 taken May 05 Valle Green	2A1502
12	5	2	A	8-Jul-05	#05020503 Section 2 taken May 05 Valle Green	2A1503
13	7	2	A	9-Sep-05	#05020504 Section 2 taken July 05 Valle Green	2A2
14	6	2	A	9-Sep-05	#05020505 Section 2 taken July 05 WPAFB cell#1	2A2
15	4	1	A	26-May-05	#05020502 Section 1 taken May 05 WPAFB cell#1	2A225
16	5	2	A	8-Jul-05	#05020503 Section 2 taken May 05 Valle Green	2A2503
17	7	3	A	9-Sep-05	#05020504 Section 3 taken July 05 Valle Green	3A1
18	6	3	A	9-Sep-05	#05020505 Section 3 taken July 05 WPAFB cell#1	3A1
19	5	3	A	8-Jul-05	#05020503 Section 3 taken May 05 Valle Green	3A1503
20	7	3	A	9-Sep-05	#05020504 Section 3 taken July 05 Valle Green	3A2
21	6	3	A	9-Sep-05	#05020505 Section 3 taken July 05 WPAFB cell#1	3A2
22	5	3	A	8-Jul-05	#05020503 Section 3 taken May 05 Valle Green	3A2503
23	2	2	A	13-Sep-05	#05020500 Section 2 taken Feb 05 WPAFB cell#1	A10
24	2	3	A	13-Sep-05	#05020500 Section 3 taken Feb 05 WPAFB cell#1	A11
25	2	3	A	13-Sep-05	#05020500 Section 3 taken Feb 05 WPAFB cell#1	A12
26	1	1	A	13-Sep-05	#05020501 Section 1 taken Jan 05 Valle Green	A13
27	1	1	A	13-Sep-05	#05020501 Section 1 taken Jan 05 Valle Green	A14
28	1	2	A	13-Sep-05	#05020501 Section 2 taken Jan 05 Valle Green	A15
29	4	2	A	26-May-05	#05020502 Section 1 taken May 05 WPAFB cell#1	1A154
30	1	2	A	13-Sep-05	#05020501 Section 2 taken Jan 05 Valle Green	A16
31	1	3	A	13-Sep-05	#05020501 Section 3 taken Jan 05 Valle Green	A17
32	1	3	A	13-Sep-05	#05020501 Section 3 taken Jan 05 Valle Green	A18
33	4	3	A	26-May-05	#05020502 Section 3 taken May 05 WPAFB cell#1	A5
34	4	3	A	26-May-05	#05020502 Section 3 taken May 05 WPAFB cell#1	A6
35	2	1	A	13-Sep-05	#05020500 Section 1 taken Feb 05 WPAFB cell#1	A7
36	2	1	A	13-Sep-05	#05020500 Section 1 taken Feb 05 WPAFB cell#1	A8
37	2	2	A	13-Sep-05	#05020500 Section 2 taken Feb 05 WPAFB cell#1	A9
38	4	1	A	26-May-05	#05020502 Section 1 taken May 05 WPAFB cell#1	C1225
39	4	2	A	26-May-05	#05020502 Section 2 taken May 05 WPAFB cell#1	C2154
40	4	2	A	26-May-05	#05020502 Section 2 taken May 05 WPAFB cell#1	E1225
41	4	2	A	26-May-05	#05020502 Section 2 taken May 05 WPAFB cell#1	E2225
42	4	2	A	26-May-05	#05020502 Section 2 taken May 05 WPAFB cell#1	G1225
43	4	2	A	26-May-05	#05020502 Section 2 taken May 05 WPAFB cell#1	G2225
44	4	3	A	26-May-05	#05020502 Section 3 taken May 05 WPAFB cell#1	I1
45	4	3	A	26-May-05	#05020502 Section 3 taken May 05 WPAFB cell#1	I2

46	4	3	A	26-May-05	#05020502 Section 3 taken May 05 WPAFB cell#1	J1
47	4	3	A	26-May-05	#05020502 Section 3 taken May 05 WPAFB cell#1	J2
48				28-Jun-05	same blank for core 02 and 03	K1
49		X		28-Jun-05	blank	K2
50	2	1	A	12-Oct-05		001
51	2	1	A	12-Oct-05		002
52	2	2	A	12-Oct-05		003
53	2	2	A	12-Oct-05		004
54	2	3	A	12-Oct-05		005
55	2	3	A	12-Oct-05		006
56	2	X		12-Oct-05	blank	007
57	2	X		12-Oct-05	blank	008
58	2	1	A	13-Oct-05		12
59	2	2	A	13-Oct-05		13
60	2	3	A	13-Oct-05		14
61	1	1	A	13-Oct-05		15
62	1	2	A	13-Oct-05		16
63	1	3	A	13-Oct-05		17
64	5	1	A	13-Oct-05		18
65	5	2	A	13-Oct-05		19
66	5	3	A	13-Oct-05		20
67	6	1	A	13-Oct-05		21
68	6	2	A	13-Oct-05		22
69	6	3	A	13-Oct-05		23
70	7	1	A	13-Oct-05		24
71	7	2	A	13-Oct-05		25
72	7	3	A	13-Oct-05		26
73	5	X		13-Oct-05	blank	27
74	7	1	A	6-Oct-05	05020502 Section 1	A1
75	7	1	A	6-Oct-05	05020502 Section 1	A2
76	7	2	A	6-Oct-05	05020502 Section 2	A3
77	7	2	A	6-Oct-05	05020502 Section 2	A4
78	7	1	A	6-Oct-05	05020502 Section 1	1A1064
79	8	1	A	10-Nov-05		
80	8	1	A	10-Nov-05		
81	8			10-Nov-05	did not use	
82	8	X		10-Nov-05	blank	
83	8	2	A	10-Nov-05		
84	8	2	A	10-Nov-05		
85	8			10-Nov-05	did not use	
86	8			10-Nov-05	blank	
87	8	3	A	10-Nov-05		
88	8	3	A	10-Nov-05		
89				10-Nov-05	did not use	
90	9	1	A	10-Nov-05		
91	9	1	A	10-Nov-05		
92	9			10-Nov-05	did not use	
93	9	X		10-Nov-05	blank	

94	9	2	A	10-Nov-05		
95	9	2	A	10-Nov-05		
96	9			10-Nov-05	did not use	
97	9	X		10-Nov-05	blank	
98	9	3	A	10-Nov-05		
99	9	3	A	10-Nov-05		
100				10-Nov-05	did not use	
101	X	X		10-Nov-05	water	

Table 6: PCR I.D.

Core Information (if applicable)	Core ID# 4 Section 2	Core ID# 4 Section 2	Core ID# 4 Section 1	Core ID# 4 Section 1	Core ID# 1 Section 1	Core ID# 4 Section 2	Core ID# 4 Section 2	Core ID# 4 Section 2	Core ID# 4 Section 2
Slide ID#	3	3	4	4	4	4	4	4	4
Band	X	X		X	X	X	X	X	
GEL Exp Date	15-Jul-05	15-Jul-05	18-Jul-05	18-Jul-05	18-Jul-05	18-Jul-05	18-Jul-05	18-Jul-05	18-Jul-05
Ann. Temp C	46	46	46	46	46	46	46	46	46
Control									
volume (µl)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Template DNA (µl)	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Template DNA (OLD)	E2225	E2225	1A154	2A225	C1225	C2154	E1225	E2225	G1225
Water (µl)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Master Mix (µl)	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
Reverse Primer (µl)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Reverse Primer	E533R	(f)UA1406	E533R	E533R	E533R	E533R	E533R	E533R	E533R
Forward Primer (µl)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Forward Primer	E8F	(f)E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F
DNA Template ID# (NEW)	41	41	29	15	38	39	40	41	42
PCR Experiment Date	15-Jul-05	15-Jul-05	18-Jul-05	18-Jul-05	18-Jul-05	18-Jul-05	18-Jul-05	18-Jul-05	18-Jul-05
PCR ID#	1	2	3	4	5	6	7	8	9

Core ID# 4 Section 2	Core ID# 4 Section 2	Core ID# 4 Section 3	Core ID# 4 Section 3	Core ID# 4 Section 3	Core ID# 4 Section 3	Blank	Blank	Core ID# 5 Section 3	Core ID# 5 Section 3	Core ID# 5 Section 2
4	4	5	5	5	5	5	5	5	5	5
	X							X		X
18-Jul-05	18-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
G2225	E2225	i1	i2	J1	J2	K1	K2	3A25-03	3A15-03	2A25-03
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E533R	(f)UA1406	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F
43	41	44	47	46	47	48	49	22	19	16
18-Jul-05	18-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05
10	11	12	13	14	15	16	17	18	19	20

Core ID# 5 Section 2	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 4 Section 1	Core ID# 4 Section 1	Core ID# 4 Section 2	Core ID# 4 Section 2	Core ID# 4 Section 2	Core ID# 4 Section 2
5	5	5	5	5	6	6	6	6	6	6
	X	X	X	X		X	X	X		X
28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
2A15-03	1B25-03	1B15-03	1A25-03	1A15-03	2A225	ug/10ul DNA)	ug/10ul DNA)	E1225	(0.117ug/10 ul DNA)	G1225
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F
12	8	7	6	3	15	38	39	40	41	42
28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	28-Jul-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05
21	22	23	24	25	26	27	28	29	30	31

Core ID# 5 Section 3	Core ID# 5 Section 3	Core ID# 5 Section 2	Core ID# 5 Section 2	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 4 Section 3	Core ID# 4 Section 3	Core ID# 4 Section 1
6	6	6	6	6	6	6	6	7	7	7
X	X	X	X	X	X	X	X	X	X	
4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	5.5	5.5	10.5
3A25-03	3A15-03	2A25-03	2A15-03	1B25-03	1B15-03	1A25-03	1A15-03	I1	J1	2A225
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00	5.00	0.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F
23	19	16	12	8	7	6	3	44	46	15
4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	4-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05
32	33	34	35	36	37	38	39	40	41	42

Core ID# 4 Section 1	Core ID# 4 Section 2	Core ID# 4 Section 2	Core ID# 4 Section 2	Core ID# 4 Section 3	Core ID# 4 Section 3	Core ID# 4 Section 3	Core ID# 4 Section 3	Blank	Core ID# 4 Section 1	Core ID# 4 Section 1
7	7	7	7	7	7	7	7	7	8	8
X		X	X		X		X			X
11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	12-Aug-05	12-Aug-05
46	46	46	46	46	46	46	46		46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
C1225	E1225	E2225	G1225	I1	I2	J1	J2	K1	2A225	C1225
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F
38	40	41	42	44	45	46	47	48	15	38
11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	12-Aug-05	12-Aug-05
43	44	45	46	47	48	49	50	51	52	53

Core ID# 4 Section 2	Core ID# 4 Section 2	Core ID# 4 Section 2	Core ID# 4 Section 3	Core ID# 4 Section 3	Core ID# 4 Section 3	Core ID# 4 Section 3	Blank	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 1
8	8	8	8	8	8	8	8	8	8	8
	X	X						X	X	X
12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05
46	46	46	46	46	46	46	46	46	46	46
							X			
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
E1225	E2225	G1225	I1	I2	J1	J2	K1	1A1503	1A2503	1B1503
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F
40	41	42	44	45	46	47	48	3	6	7
12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05
54	55	56	57	58	59	60	61	62	63	64

Core ID# 5 Section 1	Core ID# 5 Section 2	Core ID# 5 Section 2	Core ID# 5 Section 3	Core ID# 5 Section 3	Core ID# 6 Section 1	Core ID# 6 Section 1	Core ID# 6 Section 2	Core ID# 6 Section 2	Core ID# 6 Section 3	Core ID# 6 Section 3
8	8	8	8	8	9	9	9	9	9	9
	X	X		X					X	X
12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
1B2503	2A1503	2A2503	3A1503	3A2503	1A1	1A2	2A1	2A2	3A1	3A2
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E8F	E8F	E8F	E8F	E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
8	12	16	11	22	2	5	10	14	18	21
12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Sep-05	12-Sep-05	12-Sep-05	12-Sep-05	12-Sep-05	12-Sep-05
65	66	67	68	69	70	71	72	73	74	75

Core ID# 7 Section 1	Core ID# 7 Section 1	Core ID# 7 Section 2	Core ID# 7 Section 2	Core ID# 7 Section 3	Core ID# 7 Section 3	Blank	Core ID# 6 Section 1	Core ID# 6 Section 1	Core ID# 6 Section 2	Core ID# 6 Section 2
9	9	9	9	9	9	9	10	10	10	10
X	X	X	X	X	X					
12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	12-Aug-05	13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05
46	46	46	46	46	46	46	46	46	46	46
						X				
25.00	25.00	25.00	25.00	25.00	25.00	34.50	25.00	25.00	25.00	25.00
10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
1A1	1A2	2A1	2A2	3A1	3A2		1A1	1A2	2A1	2A2
0.00	0.00	0.00	0.00	0.00	0.00	9.50	0.00	0.00	0.00	0.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
1	4	9	13	17	20		2	5	10	14
12-Sep-05	12-Sep-05	12-Sep-05	12-Sep-05	12-Sep-05	12-Sep-05	12-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05
76	77	78	79	80	81	82	83	84	85	86

Core ID# 6 Section 3	Core ID# 6 Section 3	Core ID# 7 Section 1	Core ID# 7 Section 1	Core ID# 7 Section 2	Core ID# 7 Section 2	Core ID# 7 Section 3	Core ID# 7 Section 3	Blank	Core ID# 4 Section 1	Core ID# 4 Section 2
10	10	10	10	10	10	10	10	10	11	11
X	X	X	X	X	X	X	X			
13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	20/13/2005	20-Sep-05
46	46	46	46	46	46	46	46	46	46	46
								X		
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5		5.5	5.50
3A1	3A2	1A1	1A2	2A1	2A2	3A1	3A2		2A225	E1225
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.50	5.00	5.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
18	21	1	4	9	13	17	20		15	40
13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	13-Sep-05	20-Sep-05	20-Sep-05
87	88	89	90	91	92	93	94	95	96	97

Core ID# 4 Section 3	Core ID# 4 Section 3	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 2	Core ID# 5 Section 2	Core ID# 5 Section 3	Core ID# 5 Section 3	Core ID# 1 Section 1
11	11	11	11	11	11	11	11	11	11	11
20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
5.50	5.50	10.50	10.50	10.50	5.50	10.50	10.50	10.50	10.50	10.50
A5	A6	1A1503	1A2503	1B1503	1B2503	2A1503	2A2503	3A1503	3A2503	A13
5.00	5.00	0.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00	0.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
33	34	3	6	7	8	12	16	19	22	26
20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05
98	99	100	101	102	103	104	105	106	107	108

Core ID# 1 Section 1	Core ID# 1 Section 2	Core ID# 1 Section 2	Core ID# 1 Section 3	Core ID# 1 Section 3	Core ID# 2 Section 1	Core ID# 2 Section 1	Core ID# 2 Section 2	Core ID# 2 Section 2	Core ID# 2 Section 3	Core ID# 2 Section 3
11	11	11	11	11	11	11	11	11	11	11
20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
10.50	10.50	10.50	10.50	10.50	10.50	10.50	10.50	10.50	10.50	10.50
A14	A15	A16	A17	A18	A7	A8	A9	A10	A11	A12
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
27	28	30	31	32	35	36	37	23	24	25
20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05
109	110	111	112	113	114	115	116	117	118	119

Core ID# 6 Section 1	Core ID# 7 Section 2	Core ID# 7 Section 2	Core ID# 7 Section 3	Blank	Core ID# 2 Section 1	Core ID# 2 Section 1	Core ID# 2 Section 2	Core ID# 2 Section 2	Core ID# 2 Section 3	Core ID# 2 Section 3
11	11	11	11	11	12	12	12	12	12	12
					X	X	X	X	X	X
20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05
46	46	46	46	46	46	46	46	46	46	46
				X						
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
5.50	5.50	5.50	5.50	0.00	10.50	10.50	10.50	10.50	10.50	10.50
1A2	2A1	2A2	3A1	X	A7	A8	A9	A10	A11	A12
5.00	5.00	5.00	5.00	10.50	0.00	0.00	0.00	0.00	0.00	0.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
5	9	13	17	X	35	36	37	23	24	25
20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	20-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05
120	121	122	123	124	125	126	127	128	129	130

Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 2	Core ID# 5 Section 2	Core ID# 5 Section 3	Core ID# 5 Section 3	Blank	Core ID# 7 Section 1	Core ID# 7 Section 1
12	12	12	12	12	12	12	12	12	13	13
X	X	X	X	X	X	X	X			
22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	12-Oct-05	12-Oct-05
46	46	46	46	46	46	46	46	46	46	46
								X		
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	0	10.5	9
1A1503	1A2503	1B1503	1B2503	2A1503	2A2503	3A1503	3A2503	X	1A064	1A064
5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	10.50	0.00	1.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	E533R	E533R
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	E8F	E8F
3	6	7	8	12	16	19	22	X	78	78
22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05	11-Oct-05	11-Oct-05
131	132	133	134	135	136	137	138	139	140	141

Core ID# 7 Section 1	Core ID# 7 Section 1	Core ID# 7 Section 1	Core ID# 2 Section 1	Core ID# 2 Section 1	Core ID# 2 Section 1	Core ID# 2 Section 1	Core ID# 2 Section 1	Core ID# 2 Section 1	Core ID# 2 Section 1	Core ID# 2 Section 1
13	13	13	14	14	14	14	14	14	14	14
X	X	X			X	X	X	X		X
12-Oct-05	12-Oct-05	12-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
7	5	3	10.5	9	7	5	3	10.5	9	7
1A064	1A064	1A064								
3.50	5.50	7.50	0.00	1.50	3.50	5.50	7.50	0.00	1.50	3.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F
78	78	78	50	50	50	50	50	51	51	51
11-Oct-05	11-Oct-05	11-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05
142	143	144	145	146	147	148	149	150	151	152

Core ID# 2 Section 1	Core ID# 2 Section 1	Core ID# 2 Section 2	Core ID# 2 Section 2	Core ID# 2 Section 2	Core ID# 2 Section 2	Core ID# 2 Section 2	Core ID# 2 Section 2	Core ID# 2 Section 2	Core ID# 2 Section 2	Core ID# 2 Section 2
14	14	14	14	14	14	14	14	14	14	14
	X	X			X	X				X
13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
5	3	10.5	9	7	5	3	10.5	9	7	5
5.50	7.50	0.00	1.50	3.50	5.50	7.50	0.00	1.50	3.50	5.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F
51	51	52	52	52	52	52	53	53	53	53
12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05
153	154	155	156	157	158	159	160	161	162	163

Core ID# 2 Section 2	Core ID# 2 Section 3	Core ID# 2 Section 3	Core ID# 2 Section 3	Core ID# 2 Section 3	Core ID# 2 Section 3	Core ID# 2 Section 3	Core ID# 2 Section 3	Core ID# 2 Section 3	Core ID# 2 Section 3	Core ID# 2 Section 3
14	14	14	14	14	14	14	14	14	14	14
X		X	X	X	X	X	X	X	X	X
13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
3	10.5	9	7	5	3	10.5	9	7	5	3
7.50	0.00	1.50	3.50	5.50	7.50	0.00	1.50	3.50	5.50	7.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F
53	54	54	54	54	54	55	55	55	55	55
12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05
164	165	166	167	168	169	170	171	172	173	174

Core ID# 2	Core ID# 2	Core ID# 2	Core ID# 2	Core ID# 2	Core ID# 2	Core ID# 2	Core ID# 2	Core ID# 2	Core ID# 2	Core ID# 2 Section 1
14	14	14	14	14	14	14	14	n/a	n/a	15
X	X		X	X				n/a	n/a	X
13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	13-Oct-05	n/a	n/a	18-Oct-05
46	46	46	46	46	46	46	46	46	46	46
X	X	X	X	X	X	X	X	X	X	
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
10.5	9	7	5	3	10.5	9	7	5	3	7
0.00	1.50	3.50	5.50	7.50	0.00	1.50	3.50	5.50	7.50	3.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	(f)E8F
56	56	56	56	56	57	57	57	57	57	35
12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	12-Oct-05	18-Oct-05
175	176	177	178	179	180	181	182	183	184	185

Core ID# 2 Section 1	Core ID# 2 Section 2	Core ID# 2 Section 2	Core ID# 2 Section 3	Core ID# 2 Section 3	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 1	Core ID# 5 Section 2	Core ID# 5 Section 2	Core ID# 5 Section 3
15	15	15	15	15	15	15	15	15	15	15
	X	X				X	X		X	X
18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
7	7	7	7	7	7	7	7	7	7	7
3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
36	37	23	24	25	6	7	8	11	12	19
18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05	18-Oct-05
186	187	188	189	190	191	192	193	194	195	196

Core ID# 5 Section 3	Core ID# 6 Section 1	Core ID# 6 Section 1	Core ID# 6 Section 2	Core ID# 6 Section 2	Core ID# 6 Section 3	Core ID# 6 Section 3	Core ID# 1 Section 1	Core ID# 1 Section 1	Core ID# 1 Section 2	Core ID# 1 Section 2
15	16	16	16	16	16	16	16	16	16	16
18-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
7	7	7	7	7	7	7	7	7	7	7
3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
22	2	5	10	14	18	21	26	27	28	30
18-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05	24-Oct-05
197	198	199	200	201	202	203	204	205	206	207

Core ID# 1 Section 3	Core ID# 1 Section 3	Blank	Core ID# 6 Section 1	Core ID# 6 Section 1	Core ID# 6 Section 2	Core ID# 6 Section 2	Core ID# 6 Section 3	Core ID# 6 Section 3	Core ID# 1 Section 1	Core ID# 1 Section 1
16	16	16	17	17	17	17	17	17	17	17
			X	X					X	
24-Oct-05	24-Oct-05	24-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05
46	46	X	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
7	7	0	3	3	3	3	3	3	3	3
3.50	3.50	9.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
12.50	12.50	13.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
31	32	H2O	2	5	10	14	18	21	26	27
24-Oct-05	24-Oct-05	24-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05
208	209	210	211	212	213	214	215	216	217	218

Core ID# 1 Section 2	Core ID# 1 Section 2	Core ID# 1 Section 3	Core ID# 1 Section 3	Blank	Core ID# 1 Section 1	Core ID# 1 Section 2	Core ID# 1 Section 3	Core ID# 5 Section 1	Core ID# 5 Section 2	Core ID# 5 Section 3
17	17	17	17	17	18	18	18	18	18	18
		X	X		X		X		X	
25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05
46	46	46	46	X	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
3	3	3	3	0	5	5	5	5	5	5
7.50	7.50	7.50	7.50	9.50	5.50	5.50	5.50	5.50	5.50	5.50
12.50	12.50	12.50	12.50	13.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	E533R	E533R	E533R	E533R	E533R	E533R
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	E8F	E8F	E8F	E8F	E8F	E8F
28	30	31	32	H2O	61	62	63	64	65	66
25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05	25-Oct-05
219	220	221	222	223	224	225	226	227	228	229

Core ID# 5	Blank	Core ID# 1 Section 1	Core ID# 1 Section 2	Core ID# 1 Section 3	Core ID# 5 Section 1	Core ID# 5 Section 2	Core ID# 5 Section 3	Core ID# 6 Section 1	Core ID# 6 Section 2	Core ID# 6 Section 3
18	18	19	19	19	19	19	19	19	19	19
		X			X			X	X	X
25-Oct-05	25-Oct-05	27-Oct-05	27-Oct-05	27-Oct-05	27-Oct-05	27-Oct-05	27-Oct-05	27-Oct-05	27-Oct-05	27-Oct-05
46	46	46	46	46	46	46	46	46	46	46
X	X									
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
5	0	5	5	5	5	5	5	5	5	5
5.50	10.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R	E533R
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F	E8F
73	H2O	61	62	63	64	65	66	67	68	69
25-Oct-05	25-Oct-05	26-Oct-05	26-Oct-05	26-Oct-05	26-Oct-05	26-Oct-05	26-Oct-05	26-Oct-05	26-Oct-05	26-Oct-05
230	231	232	233	234	235	236	237	238	239	240

Blank	Core ID# 5	Core ID#9 Section 1	Core ID#9 Section 1		Core ID#9 Section 1	Core ID#9 Section 1		Core ID#9 Section 1	Core ID#9 Section 1	
19	19	23	23	23	23	23	23	23	23	23
						X			X	
27-Oct-05	27-Oct-05	14-Nov-05	14-Nov-05	14-Nov-05	14-Nov-05	14-Nov-05	14-Nov-05	14-Nov-05	14-Nov-05	14-Nov-05
46	46	46	46	46	46	46	46	46	46	46
X	X									
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
0	5	5	5	5	5	5	5	5	5	5
10.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E533R	E533R	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E8F	E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
H2O	73	90	91	93	94	95	97	98	99	101
26-Oct-05	26-Oct-05	14-Nov-05	14-Nov-05	14-Nov-05	14-Nov-05	14-Nov-05	14-Nov-05	14-Nov-05	14-Nov-05	14-Nov-05
241	242	290	291	292	293	294	295	296	297	298

Core ID#1 Section 1	Core ID#1 Section 1	Core ID#8 Section 1	Core ID#8 Section 1	blank	Core ID#8 Section 2	Core ID#8 Section 2	Core ID#8 Section 3	Core ID#8 Section 3	Core ID#9 Section 1	Core ID#9 Section 1
X	X	24	24	24	24	24	24	24	37	37
X	X									
15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	16-Nov-05	16-Nov-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
5	5	5	5	5	5	5	5	5	0	0
5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	10.50	10.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
26	27	79	80	82	83	84	87	88	90	91
14-Nov-05	14-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05
299	300	301	302	303	304	305	306	307	308	309

blank	Core ID#9 Section 2	Core ID#9 Section 2	Core ID#9 Section 3	Core ID#9 Section 3	Core ID#8 Section 1	Core ID#8 Section 1	blank	Core ID#8 Section 2	Core ID#8 Section 2	Core ID#8 Section 3
37	37	37	37	37	25	25	25	25	25	25
					X	X		X	X	X
16-Nov-05	16-Nov-05	16-Nov-05	16-Nov-05	16-Nov-05	17-Nov-05	17-Nov-05	17-Nov-05	17-Nov-05	17-Nov-05	17-Nov-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
5	0	5	0	5	3	3	3	3	3	3
5.50	10.50	5.50	10.50	5.50	7.50	7.50	7.50	7.50	7.50	7.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
93	94	95	98	99	79	80	82	83	84	87
15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	15-Nov-05	17-Nov-05	17-Nov-05	17-Nov-05	17-Nov-05	17-Nov-05	17-Nov-05
310	311	312	313	314	315	316	317	318	319	320

Core ID#8 Section 3	Core ID#1 Section 2	Core ID#7 Section 1	blank	Core ID#1 Section 3	Core ID#7 Section 1	Core ID#8 Section 3	Core ID#9 Section 3	Core ID#9 Section 1	Core ID#9 Section 1	Core ID#9 Section 1
25	26	26	26	26	26	26	26	27	27	27
X	X	X		X	X	X				
17-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
3	3	3	3	3	3	3	3	3	3	3
7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
88	28	80	82	31	84	87	88	90	91	93
17-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05
321	322	323	324	325	326	327	328	329	330	331

Core ID#6 Section 2	Core ID#9 Section 2	Core ID#6 Section 2	Core ID#9 Section 3	Core ID#9 Section 1	Core ID#9 Section 1	Core ID#9 Section 2	Core ID#6 Section 3	Core ID#9 Section 2	Core ID#9 Section 3	Core ID#8 Section 3
27	27	27	27	27	27	27	27	27	27	27
X	X	X	X				X	X	X	X
18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
3	3	3	3	3	3	3	3	3	3	3
7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
68	95	68	99	90	91	93	68	95	98	99
18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05	18-Nov-05
332	333	334	335	336	337	338	339	340	341	342

Core ID#8 Section 1	Core ID#8 Section 1	Core ID#8 Section 1	Core ID#6 Section 3	Core ID#9 Section 1	Core ID#8 Section 1	Core ID#1 Section 2	Core ID#9 Section 2	Core ID#8 Section 2	Core ID#2 Section 1	Core ID#2 Section 1
28	28	28	28	28	28	28	28	28	38	38
		X	X		X	X			X	X
28-Nov-05	28-Nov-05	28-Nov-05	28-Nov-05	28-Nov-05	28-Nov-05	28-Nov-05	28-Nov-05	28-Nov-05	16-Dec-05	16-Dec-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
3	5	10.5	3	2	10.5	3	2	3	2.5	2.5
7.50	5.50	0.00	7.50	8.50	0.00	7.50	8.50	7.50	8.00	8.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
79	80	79	68	90	79	27	95	83	28	29
28-Nov-05	28-Nov-05	28-Nov-05	28-Nov-05	28-Nov-05	28-Nov-05	28-Nov-05	28-Nov-05	28-Nov-05	16-Dec-05	16-Dec-05
343	344	345	346	347	348	349	350	351	352	353

Core ID#2 Section 3	Core ID#2 Section 3	Core ID#4 Section 1	Core ID#4 Section3	Core ID#4 Section 3	Core ID#6 Section 2	Core ID#6 Section 2	Core ID#8 Section 2	Core ID#7Section 1	Core ID#7 Section 1	Core ID#7 Section 2
38	38	38	38	38	38	38	38	30	30	30
X	X	X	X	X	X	X	X	X		X
16-Dec-05	16-Dec-05	16-Dec-05	16-Dec-05	16-Dec-05	16-Dec-05	16-Dec-05	16-Dec-05	29-Dec-05	29-Dec-05	29-Dec-05
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	3	3	3
8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	7.50	7.50	7.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
31	32	38	47	47	18	80	83	74	75	76
16-Dec-05	16-Dec-05	16-Dec-05	16-Dec-05	16-Dec-05	16-Dec-05	16-Dec-05	16-Dec-05	29-Dec-05	29-Dec-05	29-Dec-05
354	355	356	357	358	359	360	361	362	363	364

Core ID#7 Section 2	Core ID#8 Section 3	Core ID#8 Section 3	Core ID#7 Section 3	Core ID#7 Section 3	Core ID#4 Section 3	Core ID#8 Section 3	Core ID#8 Section 3	Core ID#8 Section 3	Core ID#2Section 1	Core ID#2 Section 1
30	30	30	31	31	31	31	31	31	32	32
	X	X	X	X	X		X	X		X
29-Dec-05	29-Dec-05	29-Dec-05	3-Jan-06	3-Jan-06	3-Jan-06	3-Jan-06	3-Jan-06	3-Jan-06	11-Jan-06	11-Jan-06
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
3	3	3	3	3	3	3	3	3	3	3
7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
77	80	80	17	17	47	80	88	88	50	50
29-Dec-05	29-Dec-05	29-Dec-05	3-Jan-06	3-Jan-06	3-Jan-06	3-Jan-06	3-Jan-06	3-Jan-06	11-Jan-06	11-Jan-06
365	366	367	368	369	370	371	372	373	374	375

Core ID#2 Section 1	Core ID#2 Section 2	Core ID#2 Section 2	Core ID#2 Section 3	Core ID#2 Section 3	Core ID#6 Section 1	Core ID#6 Section 2	Core ID#6 Section 2	Core ID#6 Section 3	Core ID#6 Section 3	Core ID#6 Section 1
32	32	32	32	32	32	32	32	32	32	32
X	X	X	X	X	X	X	X	X	X	X
11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
3	3	3	3	3	3	3	3	3	3	5
7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	5.50
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
50	52	52	54	54	2	68	68	69	69	2
11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06
376	377	378	379	380	381	382	383	384	385	386

Core ID#4 Section 1	Core ID#4 Section 2	Core ID#4 Section 2	Core ID#4 Section 3	Core ID#4 Section 3	Core ID#7 Section 1	Core ID#7 Section 1	Core ID#7 Section 2	Core ID#7 Section 2	Core ID#7 Section 3	Core ID#7 Section 3
32	32	32	32	32	33	33	33	33	33	33
X					X	X	X	X	X	X
11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
5	5	5	5	5	2.5	2.5	2.5	2.5	2.5	2.5
5.50	5.50	5.50	5.50	5.50	8.00	8.00	8.00	8.00	8.00	8.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
38	43	43	44	44	4	4	9	9	17	17
11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	11-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06
387	388	389	390	391	392	393	394	395	396	397

Core ID#1 Section 1	Core ID#1 Section 1	Core ID#1 Section 2	Core ID#1 Section 2	Core ID#1 Section 3	Core ID#1 Section 3	Core ID#9 Section 1	Core ID#9 Section 1	Core ID#9 Section 2	Core ID#9 Section 2	Core ID#9 Section 3
33	33	33	33	33	33	34	34	34	34	34
X	X	X	X	X	X	X	X	X	X	X
13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
26	26	30	30	31	31	90	90	94	94	98
13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06
398	399	400	401	402	403	404	405	406	407	408

Core ID#9 Section 3	Core ID#5 Section 1	Core ID#5 Section 1	Core ID#5 Section 2	Core ID#5 Section 2	Core ID#5 Section 3	Core ID#5 Section 3	Core ID#1Section 1	Core ID#1 Section 1	Core ID#1 Section 2	Core ID#1 Section 2
34	34	34	34	34	34	34	35	35	35	35
X	X	X	X	X	X	X	X	X	X	X
19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
98	6	6	11	11	19	19	26	26	30	30
18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06
409	410	411	412	413	414	415	416	417	418	419

Core ID#1 Section 3	Core ID#1 Section 3	Core ID#1 Section 3	Core ID#1 Section 3	Core ID#7 Section 1	Core ID#7 Section 1	Core ID#7 Section 2	Core ID#7 Section 2	Core ID#7 Section 3	Core ID#7 Section 3	Core ID#7 Section 3
35	35	35	35	35	35	35	35	35	35	35
X	X	X	X	X	X	X	X	X	X	X
19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
31	31	31	31	4	4	9	9	17	17	17
19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06
420	421	422	423	424	425	426	427	428	429	430

Core ID#7 Section 3	Core ID#2 Section 1	Core ID#2 Section 1	Core ID#2 Section 2	Core ID#2 Section 2	Core ID#2 Section 3	Core ID#2 Section 3	Core ID#6 Section 1	Core ID#6 Section 1	Core ID#6 Section 2	Core ID#6 Section 2
35	36	36	36	36	36	36	36	36	36	36
X			X	X	X	X	X	X	X	X
19-Jan-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
2.5	3	3	3	3	3	3	4	4	4	4
8.00	7.50	7.50	7.50	7.50	7.50	7.50	6.5	6.5	6.5	6.5
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
17	50	50	52	52	54	54	40	40	38	38
19-Jan-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06	28-Feb-06
431	432	433	434	435	436	437	438	439	440	441

Core ID#6 Section 3	Core ID#6 Section 3	Core ID#2 Section 2	Core ID#2 Section 2	Core ID#5 Section 2	Core ID#5 Section 2	Core ID#7 Section 2	Core ID#2 Section 2	Core ID#2 Section 2	Core ID#7 Section 2	Core ID#7 Section 2
36	36	39	39	39	39	39	39	39	39	39
X	X	X	X	X	X	X	X	X	X	X
28-Feb-06	28-Feb-06	10-Mar-06	10-Mar-06	10-Mar-06	10-Mar-06	10-Mar-06	10-Mar-06	10-Mar-06	10-Mar-06	10-Mar-06
46	46	46	46	46	46	46	46	46	46	46
25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
4	4	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406	(f)UA1406
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F	(f)E8F
44	44	52	11	11	9	52	52	52	9	9
28-Feb-06	28-Feb-06	10-Mar-06	10-Mar-06	10-Mar-06	10-Mar-06	10-Mar-06	10-Mar-06	10-Mar-06	10-Mar-06	10-Mar-06
442	443	444	445	446	447	448	449	450	451	452

453	10-Mar-06	11	(f)E8F	1.00	(f)UA1406	1.00	12.50	6.5		2.5	25.00		46		X	39	Core ID#5 Section 2
454	10-Mar-06	11	(f)E8F	1.00	(f)UA1406	1.00	12.50	6.5		2.5	25.00		46	10-Mar-06	X	39	Core ID#5 Section 2
455	10-Mar-06	9	(f)E8F	1.00	(f)UA1406	1.00	12.50	6.5		2.5	25.00		46	10-Mar-06	X	39	Core ID#7 Section 2

Table 7: Percentage of DNA concentration

340 raw	0.78	0.769	0.79	0.778	0.782	0.77	0.784	0.773	0.77
Cursor abs.	1.001	0.978	1.22	1.639	1.787	1.025	1.085	1.044	1.072
Cursor Pos.	230	230	230	230	230	230	230	230	230
Constant	50	50	50	50	50	50	50	50	50
260/230	1.12	1.11	1.52	0.48	0.73	1.25	1.38	1.17	1.23
260/280	1.9	1.93	1.94	1.77	1.84	1.87	1.85	1.76	1.9
A280	0.587	0.56	0.958	0.445	0.704	0.686	0.808	0.695	0.693
A260	1.118	1.081	1.854	0.788	1.299	1.285	1.496	1.223	1.315
ng/ul	55.91	54.06	92.69	39.4	64.93	64.27	74.78	61.16	65.75
Time	4:21 PM	4:25 PM	4:27 PM	4:28 PM	4:29 PM	4:30 PM	4:31 PM	4:33 PM	4:34 PM
Date	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006
User ID	Default	Default	Default	Default	Default	Default	Default	Default	Default
Sample ID	326	326	362	79	80	368	369	315	316

0.755	0.741	0.776	0.784	0.767	0.776	0.757	0.785	0.756	0.77	0.773
0.691	0.7	1.081	1.088	0.778	1.113	0.784	0.807	0.756	0.822	0.847
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
0.67	0.68	1.29	1.3	0.89	1.3	0.91	0.77	0.84	0.87	0.95
1.49	1.4	1.99	1.99	1.85	1.96	1.87	1.92	1.82	1.69	1.85
0.313	0.339	0.7	0.711	0.377	0.738	0.381	0.322	0.349	0.421	0.436
0.466	0.475	1.395	1.415	0.695	1.446	0.715	0.619	0.635	0.713	0.809
23.28	23.74	69.77	70.76	34.76	72.29	35.74	30.96	31.76	35.66	40.44
4:35 PM	4:36 PM	4:37 PM	4:38 PM	4:39 PM	4:40 PM	4:41 PM	4:43 PM	4:44 PM	4:45 PM	4:46 PM
1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
345	348	318	319	342	327	297	274	363	333	341

0.785	0.778	0.754	0.788	0.723	0.76	0.781	0.771	0.771	0.828	0.779
0.845	0.879	1.04	0.969	1.022	1.045	0.623	0.861	0.645	0.763	1.053
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
0.96	1.03	1.04	1.31	1.3	1.13	0.61	1.02	0.6	0.61	1.11
1.87	1.81	1.8	1.93	1.72	1.77	1.9	1.85	1.57	1.6	1.84
0.435	0.5	0.597	0.659	0.772	0.665	0.201	0.475	0.245	0.29	0.633
0.814	0.903	1.077	1.27	1.329	1.18	0.381	0.878	0.386	0.462	1.164
40.68	45.17	53.84	63.52	66.44	58.98	19.06	43.89	19.3	23.12	58.18
4:47 PM	4:49 PM	4:50 PM	4:51 PM	4:52 PM	4:53 PM	4:54 PM	4:55 PM	4:56 PM	4:57 PM	4:58 PM
1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
335	337	339	372	373	323	346	332	290	291	75

0.788	0.917	0.779	0.782	0.792	0.771	0.794	12.088	1.971	0.008	-0.041
1.608	1.049	0.954	1.074	0.859	0.993	1.022	2.982	0.442	0.032	0.056
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
0.53	1.11	0.89	0.97	0.84	0.9	0.89	0.85	0.68	9.33	7.21
1.83	1.85	1.79	1.81	1.66	1.74	1.78	1.1	2.43	2.3	2.03
0.465	0.629	0.473	0.576	0.433	0.516	0.513	2.309	0.123	0.128	0.2
0.852	1.161	0.85	1.042	0.718	0.896	0.914	2.53	0.299	0.294	0.404
42.6	58.04	42.48	52.11	35.88	44.82	45.68	126.49	14.96	14.7	20.21
5:00 PM	5:01 PM	5:03 PM	5:03 PM	5:05 PM	5:06 PM	5:06 PM	5:07 PM	3:04 PM	3:05 PM	3:06 PM
1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/11/2006	1/17/2006	1/17/2006	1/17/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
193	62	64	127	63	53	53	55	374	375	376

28.956	-0.004	-0.005	-0.019	-0.017	0.024	-0.028	-0.012	-0.016	-0.003	-0.007
0.968	0.045	0.036	0.044	-0.025	-0.033	0.057	0.043	0.047	0.011	0.1
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
0.98	8.18	9.02	6.49	-9.24	-6.5	5.04	7.74	7.83	30.05	2.39
1.59	2.27	2.13	1.9	2.44	2.4	2.36	2.18	2.2	2.14	2.09
0.598	0.163	0.151	0.151	0.094	0.09	0.121	0.152	0.167	0.148	0.115
0.949	0.37	0.323	0.288	0.23	0.215	0.286	0.331	0.368	0.317	0.24
47.43	18.51	16.16	14.42	11.5	10.76	14.28	16.55	18.4	15.85	12.02
3:06 PM	3:07 PM	3:08 PM	3:09 PM	3:10 PM	3:11 PM	3:11 PM	3:12 PM	3:13 PM	3:14 PM	3:16 PM
1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
377	377	378	379	380	381	382	383	384	385	386

-0.006	-0.013	-0.018	0.005	0.001	-0.024	-0.017	-0.035	-0.01	-0.009	-0.002
0.476	-0.019	-0.027	0.153	-0.001	0.011	-0.017	0.041	-0.013	-0.036	-0.035
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
0.56	-13.89	-7.62	3.62	-297.19	30.28	-16.74	7.24	-17.83	-4.77	-5.21
2.07	2.21	2.24	2.03	1.98	2.21	2.09	1.97	2.17	1.67	2.18
0.128	0.119	0.091	0.273	0.116	0.145	0.132	0.152	0.105	0.103	0.084
0.266	0.262	0.205	0.552	0.23	0.321	0.277	0.3	0.228	0.171	0.182
13.29	13.09	10.24	27.6	11.51	16.07	13.86	14.99	11.4	8.54	9.09
3:16 PM	3:17 PM	3:18 PM	3:18 PM	3:19 PM	3:20 PM	3:20 PM	3:21 PM	3:22 PM	3:22 PM	3:23 PM
1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
387	388	389	390	391	392	393	394	395	396	397

-0.001	-0.031	-0.022	-0.015	-0.023	-0.031	0.006	-0.003	0.015	-0.001	-0.04
-0.056	0.009	0.01	-0.032	0.068	0.003	0.007	0.071	0.136	0.315	-0.084
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
-2.78	34.38	27.4	-5.94	4.99	97.73	30.95	4.5	3.85	2.56	-0.09
2.7	1.81	2.61	2.49	1.78	2.07	2.04	1.93	1.93	1.91	0.51
0.058	0.171	0.106	0.076	0.19	0.156	0.104	0.166	0.272	0.424	0.015
0.156	0.31	0.276	0.19	0.338	0.323	0.212	0.319	0.523	0.808	0.008
7.79	15.48	13.81	9.51	16.91	16.13	10.6	15.97	26.17	40.39	0.38
3:24 PM	3:25 PM	3:26 PM	3:26 PM	3:27 PM	3:28 PM	3:29 PM	3:29 PM	3:30 PM	3:31 PM	3:32 PM
1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
396	398	399	400	401	401	402	403	217	43	2

-0.02	-0.013	0.003	-0.019	0.004	-0.036	0.043	0	0.041	-0.017	-0.006
-0.086	0.222	-0.038	0.177	0.645	0.252	0.218	0.287	0.076	0.046	0.324
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
-0.05	2.55	-5.47	2.36	0.41	2.45	0.87	0.89	4.35	2.41	2.26
0.64	1.81	2.41	1.82	1.67	1.47	15.32	1.75	2.06	2.11	1.87
0.006	0.312	0.086	0.23	0.159	0.42	0.012	0.145	0.161	0.053	0.391
0.004	0.566	0.206	0.418	0.266	0.619	0.189	0.255	0.331	0.112	0.73
0.2	28.31	10.32	20.91	13.29	30.93	9.46	12.73	16.56	5.59	36.51
3:32 PM	3:33 PM	3:34 PM	3:35 PM	3:36 PM	3:37 PM	3:38 PM	3:39 PM	3:39 PM	3:41 PM	3:42 PM
1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
2	224	66	67	192	87	196	196	77	76	81

-0.022	-0.017	0	0	-0.013	-0.015	0.002	0.778	0.669	0.79	0.758
0.157	0.629	-0.04	-0.046	0.178	-0.014	-0.023	1.001	0.988	1.23	1.64
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
3.28	0.79	2.11	2.06	3.31	-6.06	-3.8	1.13	1.11	1.53	0.48
1.83	1.99	3.22	1.76	1.9	1.49	2.03	1.94	1.92	1.96	1.72
0.282	0.25	-0.026	-0.054	0.309	0.058	0.044	0.588	0.569	0.958	0.446
0.515	0.497	-0.085	-0.096	0.588	0.087	0.089	1.119	1.081	1.855	0.788
25.74	24.86	-4.24	-4.8	29.38	4.35	4.44	55.81	53.16	93.67	39.87
3:43 PM	3:43 PM	3:45 PM	3:46 PM	3:47 PM	3:48 PM	3:48 PM	4:22 PM	4:25 PM	4:27 PM	4:28 PM
1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
89	90	91	91	92	94	94	299	300	322	325

0.7682	0.977	0.874	0.773	0.67	0.855	0.751	0.776	0.684	0.867	0.976
1.787	1.022	1.085	1.054	1.073	0.691	0.8	1.081	1.098	0.778	1.125
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
0.75	1.26	1.38	1.18	1.24	0.67	0.69	1.28	1.31	0.88	1.3
1.83	1.86	1.83	1.77	1.98	1.48	1.46	1.97	1.93	1.84	1.915
0.704	0.681	0.808	0.692	0.689	0.323	0.339	0.79	0.712	0.3727	0.739
1.29	1.285	1.491	1.223	1.317	0.462	0.475	1.392	1.415	0.693	1.446
64.69	66.3	73.69	62.21	64.75	22.27	22.69	70.77	70.19	34.25	73
4:29 PM	4:30 PM	4:31 PM	4:32 PM	4:34 PM	4:35 PM	4:36 PM	4:37 PM	4:38 PM	4:39 PM	4:40 PM
1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
352	353	354	355	356	364	45	56	357	370	131

0.737	0.745	0.656	0.775	0.773	0.795	0.718	0.724	0.783	0.723	0.86
0.784	0.907	0.756	1.002	0.847	0.945	0.879	1.04	0.0882	1.022	1.23
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
0.96	0.78	0.85	0.87	0.96	0.98	1.03	1.05	1.31	1.32	1.14
1.83	1.95	1.82	1.67	1.86	1.83	1.82	1.8	1.95	1.74	1.72
0.381	0.323	0.349	0.429	0.437	0.432	0.55	0.5957	0.659	0.782	0.667
0.713	0.619	0.637	0.713	0.807	0.814	0.907	1.077	1.272	1.329	1.185
35.15	30.85	31.27	35.19	40.97	39.97	45.91	52.92	63.09	66.96	58.91
4:41 PM	4:42 PM	4:44 PM	4:45 PM	4:46 PM	4:48 PM	4:49 PM	4:50 PM	4:51 PM	4:52 PM	4:53 PM
1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
132	325	349	195	69	137	196	211	212	332	346

0.721	0.731	0.671	0.818	0.779	0.788	0.917	0.867	0.882	0.892	0.771
0.723	0.879	0.645	0.835	1.053	1.765	1.049	0.978	1.074	0.983	0.993
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
0.61	1.04	0.62	0.61	1.12	0.53	1.13	0.89	0.98	0.85	0.91
1.91	1.87	1.59	1.61	1.86	1.83	1.87	1.79	1.83	1.64	1.74
0.202	0.475	0.246	0.299	0.633	0.464	0.628	0.472	0.572	0.433	0.517
0.381	0.879	0.386	0.465	1.164	0.857	1.161	0.83	1.043	0.7168	0.896
20.9	44.2	19.3	23.89	58.87	42.61	58.51	42.2	52.89	35.2	44.19
4:54 PM	4:55 PM	4:56 PM	4:57 PM	4:58 PM	5:00 PM	5:01 PM	5:03 PM	5:04 PM	5:05 PM	5:06 PM
1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
334	339	326	362	80	368	369	315	316	318	319

0.754	11.088	1.871	0.008	-0.061	27.956	-0.008	-0.006	-0.022	-0.027	0.024
1.03	2.765	0.448	0.0334	0.0066	0.968	0.055	0.0478	0.074	-0.03	-0.0453
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
0.89	0.86	0.68	9.34	7.21	0.97	8.18	9.03	6.49	-9.25	-6.5
1.78	1.12	2.47	2.4	2.01	1.59	2.25	2.16	1.91	2.45	2.42
0.511	2.31	0.126	0.128	0.29	0.592	0.169	0.153	0.154	0.095	0.096
0.917	2.53	0.298	0.294	0.402	0.949	0.375	0.323	0.282	0.231	0.215
45.25	126.92	14	14.71	20.99	47.71	18.12	16.86	14.15	11.51	10.12
5:07 PM	5:07 PM	5:08 PM	5:09 PM	5:10 PM	5:11 PM	5:12 PM	5:13 PM	5:14 PM	5:15 PM	5:16 PM
1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
327	341	335	75	339	372	373	404	405	406	407

408	Default	1/17/2006	5:17 PM	14.79	0.287	0.127	2.36	5.06	50	230	0.057	-0.038
409	Default	1/17/2006	5:18 PM	16.51	0.332	0.156	2.18	7.77	50	230	0.067	-0.022
410	Default	1/17/2006	5:18 PM	18.49	0.3679	0.167	2.22	7.83	50	230	0.051	-0.027
411	Default	1/17/2006	5:19 PM	15.1	0.317	0.143	2.15	30.07	50	230	0.0198	-0.005
412	Default	1/17/2006	5:20 PM	12.14	0.246	0.115	2.08	2.39	50	230	0.119	-0.009
413	Default	1/17/2006	5:21 PM	13.2	0.268	0.124	2.08	0.57	50	230	0.532	-0.007
414	Default	1/17/2006	5:22 PM	13.09	0.263	0.117	2.21	-13.88	50	230	-0.028	-0.023
415	Default	1/17/2006	5:23 PM	10.32	0.205	0.093	2.26	-7.62	50	230	-0.3253	-0.028
416	Default	1/17/2006	5:24 PM	27.63	0.553	0.273	2.04	3.65	50	230	0.1345	0.004
417	Default	1/17/2006	5:24 PM	11.54	0.239	0.114	1.99	-297.15	50	230	-0.00234	0.002
418	Default	1/17/2006	5:25 PM	16.05	0.321	0.145	2.23	30.28	50	230	0.011	-0.034

-0.027	-0.045	-0.02	-0.008	-0.002	-0.003	-0.034	-0.024	-0.017	-0.033	-0.032
-0.0234	0.0624	-0.0235	-0.036	-0.047	-0.065	0.0012	0.002	-0.023	0.086	0.0047
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
-16.77	7.24	-17.85	-4.77	-5.24	-2.789	34.38	27.7	-5.95	4.99	97.75
2.12	1.95	2.18	1.68	2.16	2.7	1.83	2.63	2.45	1.76	2.08
0.138	0.152	0.107	0.103	0.084	0.058	0.176	0.106	0.077	0.19	0.156
0.276	0.3	0.229	0.171	0.183	0.156	0.32	0.277	0.195	0.3379	0.3229
13.78	14.9	11.44	8.56	9.09	7.73	15.45	13.82	9.59	16.93	16.13
5:26 PM	5:27 PM	5:28 PM	5:29 PM	5:30 PM	5:31 PM	5:32 PM	5:33 PM	5:33 PM	5:34 PM	5:35 PM
1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006	1/17/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
419	420	421	422	423	424	425	426	427	428	429

0.006	0.0065	230	50	30.98	2.05	0.105	0.212	10.65	5:36 PM	1/17/2006	Default	430
-0.005	0.076	230	50	4.7	1.94	0.166	0.318	15.97	5:37 PM	1/17/2006	Default	431
0.0165	0.136	230	50	3.87	1.96	0.274	0.524	26.22	5:39 PM	1/17/2006	Default	432
-0.003	0.297	230	50	2.56	1.91	0.424	0.808	40.35	5:40 PM	1/17/2006	Default	433
0.014	5.163	230	50	1.15	1.97	3.011	5.923	296.14	8:46 AM	3/13/2006	Default	432
0.032	5.919	230	50	1.29	2	3.827	7.657	382.83	8:49 AM	3/13/2006	Default	433
0.019	6.494	230	50	1.36	1.96	4.513	8.854	442.71	8:50 AM	3/13/2006	Default	436
0.101	7.191	230	50	1.35	1.98	4.907	9.725	486.26	8:51 AM	3/13/2006	Default	437
0.182	7.936	230	50	1.25	1.98	5.016	9.924	496.21	8:53 AM	3/13/2006	Default	438
0.166	7.697	230	50	1.21	1.95	4.777	9.326	466.28	8:54 AM	3/13/2006	Default	438

0.014	0.025	0.032	0.014	0.039	0.051	0.051	0.101	0.129	-0.585	0.083
6.57	6.32	6.26	6.18	3.933	4.111	4.371	4.502	4.798	4.173	4.321
230	230	230	230	230	230	230	230	230	230	230
50	50	50	50	50	50	50	50	50	50	50
1.47	1.40	1.36	1.28	1.98	2.03	1.99	1.96	1.91	2.02	2.02
1.96	1.86	1.82	1.76	1.94	1.93	1.96	1.92	1.93	1.89	1.87
4.938	4.790	4.689	4.532	4.014	4.314	4.485	4.534	4.737	4.344	4.508
9.641	9.256	9.129	9.05	7.805	8.347	8.704	8.811	9.169	8.432	8.736
482.04	462.04	452.10	431.56	390.24	417.35	435.21	440.56	458.44	421.58	436.8
8:56 AM	8:56 AM	8:56 AM	8:56 AM	8:57 AM	8:59 AM	9:00 AM	9:01 AM	9:02 AM	9:03 AM	9:04 AM
3/13/2006	3/13/2006	3/13/2006	3/13/2006	3/13/2006	3/13/2006	3/13/2006	3/13/2006	3/13/2006	3/13/2006	3/13/2006
Default	Default	Default	Default	Default	Default	Default	Default	Default	Default	Default
440	441	442	443	444	445	445	446	447	448	449

550	Default	3/13/2006	9:06 AM	453.23	9.065	4.663	1.91	2.02	50	230	4.496	0.076
551	Default	3/13/2006	9:07 AM	461.81	9.236	4.768	1.82	2.01	50	230	4.604	0.043
552	Default	3/13/2006	9:08 AM	443.11	8.862	4.56	1.86	2.04	50	230	4.351	0.082
453	Default	3/13/2006	9:09 AM	453.11	9.062	4.685	1.93	2.03	50	230	4.458	0.047
454	Default	3/13/2006	9:11 AM	455.3	9.106	4.687	1.95	2.04	50	230	4.469	0.073

Table 8: Rsa1 results

Remarks	cleaned	cleaned		cleaned	cleaned	cleaned		smeared	smeared
Slide #	1	1	1	1	2	2	2	3	3
Band size (bp)	cannot be determined	cannot be determined		cannot be determined	cannot be determined	cannot be determined		400,500	400,500
Gel %	2	2	2	2	2	2	2	2	2
GEL Experiment Date	8-Dec-05	8-Dec-05	8-Dec-05	8-Dec-05	12-Dec-05	12-Dec-05	12-Dec-05	15-Dec-05	15-Dec-05
Incubation Temp C	37	37		37	37	37		37	37
Control			X				X		
Total volume (µl)	20.5	20.5		20.5	20.5	20.5		20.5	20.5
BSA (ul)	0.5	0		0	0	0		0	0
DNA (µl)	18	18		18	18	18		18	18
Water (µl)									
Rsa I (µl)	0.5	0.5		0.5	0.5	0.5		0.5	0.5
Buffer (ul)	2	2		2	2	2		2	2
PCR ID#	138	195		88	129	185		316	327

	cleaned	cleaned		not cleaned	not cleaned	cleaned	cleaned		cleaned	cleaned
3	4	4	4	5	5	5	5	5	6	6
	500,400	500, 400		400	400	500, 450, 400, 250	450,400		cannot be determined	cannot be determined
2	2	2	2	2	2	2	2		3	3
15-Dec-05	16-Dec-05	16-Dec-05	16-Dec-05	18-Dec-05	18-Dec-05	18-Dec-05	18-Dec-05	18-Dec-05	30-Dec-05	30-Dec-05
	37	37		37	37	37	37		37	37
X			X					X		
	20.5	20.5		20.5	20.5	20.5	20.5		20.5	20.5
	0	0		0	0	0	0		0.5	0.5
	18	18		18	18	18	18		18	18
	0.5	0.5		0.5	0.5	0.5	0.5		0.5	0.5
	2	2		2	2	2	2		2	2
	360	361		360	361	127	342		320	321

not cleaned	not cleaned		bad gelbox	bad gelbox	bad gelbox	bad gelbox		cleaned	cleaned	not cleaned
6	6	6	7	7	7	7	7	8	8	8
500	500		cannot be determined	cannot be determined	cannot be determined	cannot be determined		200, 300, 400, 500	200, 300. , 400, 500	200, 300, 400, 500
3	3	3	2	2	2	2	2	2	2	2
30-Dec-05	30-Dec-05	30-Dec-05	4-Jan-06	4-Jan-06	4-Jan-06	4-Jan-06	4-Jan-06	5-Jan-06	5-Jan-06	5-Jan-06
37	37		37	37	37	37		37	37	37
		X					X			
20.5	20.5		20.5	20.5	20.5	20.5		20.5	20.5	20.5
0.5	0.5		0.5	0.5	0.5	0.5		0.5	0.5	0.5
18	18		18	18	18	18		18	18	18
0.5	0.5		0.5	0.5	0.5	0.5		0.5	0.5	0.5
2	2		2	2	2	2		2	2	2
366	367		320	321	372	373		127	128	187

not cleaned		cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned
8	8	9	9	9	9	9	9	9	9	9
200, 300, 400, 500		cannot be determined	500, 400, 350	cannot be determined	500,450,400	500,450,400	cannot be determined	500	450	400
2		2	2	2	2	2	2	2	2	2
5-Jan-06	5-Jan-06	6-Jan-06	6-Jan-06	6-Jan-06	6-Jan-06	6-Jan-06	6-Jan-06	6-Jan-06	6-Jan-06	6-Jan-06
37		37	37	37	37	37	37	37	37	37
	X									
20.5		20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
0.5		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
18		18	18	18	18	18	18	18	18	18
0.5		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2		2	2	2	2	2	2	2	2	2
188		299	300	222	325	322	349	352	353	354

cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned		cleaned	cleaned	cleaned
9	9	9	9	9	9	9	9	10	10	10
550, 400, 250, 150	400, 350, 150	cannot be determined	500, 450, 400	500, 400	550, 500, ,300	550, 500, 300		1,300, 900, 550, 450, 200	1300, 900, 550, 450, 200	1,300, 900,550, 450, 200
2	2	2	2	2	2	2	2	2.5	2.5	2.5
6-Jan-06	6-Jan-06	6-Jan-06	6-Jan-06	6-Jan-06	6-Jan-06	6-Jan-06	6-Jan-06	8-Jan-06	8-Jan-06	8-Jan-06
37	37	37	37	37	37	37		37	37	37
							X			
20.5	20.5	20.5	20.5	20.5	20.5	20.5		20.5	20.5	20.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5	0.5	0.5
18	18	18	18	18	18	18		18	18	18
0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5	0.5	0.5
2	2	2	2	2	2	2		2	2	2
355	356	364	45	56	357	370		131	132	322

cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	
10	10	10	10	10	10	10	10	10	10	10
1,300, 900,550, 450, 200	cannot be determined	1,300, 900, 550, 450, 200	1,300, 900, 550, 450, 200	1,300, 900, 550, 450, 200	cannot be determined	cannot be determined	1,300, 900, 550, 450, 200	1,300, 900, 550, 450, 200	1,300, 900, 550, 450, 200	
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
8-Jan-06	8-Jan-06	8-Jan-06	8-Jan-06	8-Jan-06	8-Jan-06	8-Jan-06	8-Jan-06	8-Jan-06	8-Jan-06	8-Jan-06
37	37	37	37	37	37	37	37	37	37	
										X
20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
18	18	18	18	18	18	18	18	18	18	
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
2	2	2	2	2	2	2	2	2	2	
325	349	352	353	354	355	356	357	364	370	

cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	
11	11	11	11	11	11	11	11	11	11	11
cannot be determined	500, 450, 400, 200	500, 450, 400, 200	cannot be determined	900, 800, 550, 500, 250, 200	900, 800, 550, 500, 250, 200	cannot be determined	900, 800, 550, 500, 300, 200	cannot be determined	900, 800, 550, 500, 300, 200	
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
9-Jan-06	9-Jan-06	9-Jan-06	9-Jan-06	9-Jan-06	9-Jan-06	9-Jan-06	9-Jan-06	9-Jan-06	9-Jan-06	9-Jan-06
37	37	37	37	37	37	37	37	37	37	
										X
20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
18	18	18	18	18	18	18	18	18	18	
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
2	2	2	2	2	2	2	2	2	2	
195	69	137	196	211	212	332	346	334	339	

cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned
				12	12	12	12	12	12	12
1400, 1300, 950, 550, 400, 200, 150	1300, 950, 550, 400, 200, 150	1300, 950, 550, 400, 2000, 150	900, 850, 550, 400, 400, 200, 150	900, 850, 550, 400, 400, 200, 150	1400, 1300, 950, 550, 400, 200, 150	1400, 1300, 950, 550, 400, 200, 150	550, 400, 200, 150	550, 400, 380, 200, 150	550, 400, 200, 150	1400, 1000, 900, 850, 550, 400, 200, 150
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
8-Jan-06	8-Jan-06	8-Jan-06	8-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06
37	37	37	37	37	37	37	37	37	37	37
20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
16	11	14	14	13	14	14	15	15	14	18
2.5	7	3.5	3.5	4.5	3.5	3.5	3	3	3.5	
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	2	2	2	2	2	2	2	2	2	2
326	362	80	368	369	315	316	318	319	327	341

cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned		cleaned	cleaned	cleaned
12	12	12	12	12	12	12	12	13	13	13
1400, 1000, 900, 850, 550, 400, 200, 150	1400, 900, 850, 550, 400, 200, 150	1400, 900, 850, 550, 400, 200, 150	550, 400, 200, 150	550, 400, 200, 150	1300, 950, 550, 400, 200, 150	1300, 950, 550, 400, 200, 150		1400, 1300, 900, 500, 400,, 200, 150	1400, 1300, 900, 500, 400, 200, 150	1300, 900, 500, 400, 200, 150
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	13-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06
37	37	37	37	37	37	37		37	37	37
							X			
20.5	20.5	20.5	20.5	20.5	20.5	20.5		20.5	20.5	20.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5	0.5	0.5
18	18	18	14	14	18	18		12	12	14
			3.5	3.5				5.5	5.5	3.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5	0.5	0.5
2	2	2	2	2	2	2		2	2	2
335	75	339	372	373	323	332		392	393	394

cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned		cleaned
13	13	13	13	13	13	13	13	13	13	14
1300, 900, 500, 400, , 200, 150	1300, 900, 500, 400, 200, 150	1300, 900, 500, 400, 200, 150	500, 400	500, 400	500, 400	500, 400	900, 800, 500, 400, 350	900, 800, 500, 400, 350		900, 800, 650, 500, 400, 350, 250, 150
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	18-Jan-06	19-Jan-06
37	37	37	37	37	37	37	37	37		37
									X	
20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5		20.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5
12	15	12	12	10	10	12	12	13		14
5.5	2.5	5.5	5.5	7.5	7.5	5.5	5.5	4.5		3
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5
2	2	2	2	2	2	2	2	2		2
395	396	397	398	399	400	401	402	403		404

cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned
14	14	14	14	14	14	14	14	14	14	14
900, 800, 650, 500, 400, 350, 250, 150	1400 (undigested) , 1000, 900, 800, 400, 300, 200	1400 (undigested) , 1000, 900, 800, 400, 300, 200	1400 (undigested) , 1000, 900, 800, 400, 300, 200	1400 (undigested) 1000, 900, 800, 400, 300, 200	cannot be determined	cannot be determined	500 and 400	500 and 400	500, 400	500, 400
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06	19-Jan-06
37	37	37	37	37	37	37	37	37	37	37
20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
14	14	14	14	14	14	14	14	14	14	14
3	3	3	3	3	3	3	3	3	3	3
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	2	2	2	2	2	2	2	2	2	2
405	406	407	408	409	410	411	412	413	414	415

	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned
14	15	15	15	15	15	15	15	15	15	15
	cannot be determined	cannot be determined	cannot be determined	cannot be determined	cannot be determined	cannot be determined	cannot be determined	cannot be determined	cannot be determined	cannot be determined
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
19-Jan-06	20-Jan-06	20-Jan-06	20-Jan-06	20-Jan-06	20-Jan-06	20-Jan-06	20-Jan-06	20-Jan-06	20-Jan-06	20-Jan-06
	37	37	37	37	37	37	37	37	37	37
X										
	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	14	14	14	14	14	14	14	14	14	14
	3	3	3	3	3	3	3	3	3	3
	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	2	2	2	2	2	2	2	2	2	2
	416	417	418	419	420	421	422	423	424	425

cleaned	cleaned	cleaned	cleaned		cleaned	cleaned	cleaned	cleaned	cleaned	cleaned
15	15	15	15	15	16	16	16	16	16	16
cannot be determined	cannot be determined	cannot be determined	cannot be determined		1400, 1300, 900, 500, 400, 200, 150	1400, 1300, 900, 500, 400, 200, 150	1300, 900, 500, 400, 200, 150	1300, 900, 500, 400, 200, 150	1300, 900, 500, 400, 200, 150	1300, 900, 500, 400, 200, 150
2.5	2.5	2.5	2.5		2.5	2.5	2.5	2.5	2.5	2.5
20-Jan-06	20-Jan-06	20-Jan-06	20-Jan-06	20-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06
37	37	37	37		37	37	37	37	37	37
				X						
20.5	20.5	20.5	20.5		20.5	20.5	20.5	20.5	20.5	20.5
0.5	0.5	0.5	0.5		0.5	0.5	0.5	0.5	0.5	0.5
14	14	14	14		12	12	14	12	15	12
3	3	3	3		5.5	5.5	3.5	5.5	2.5	5.5
0.5	0.5	0.5	0.5		0.5	0.5	0.5	0.5	0.5	0.5
2	2	2	2		2	2	2	2	2	2
426	427	428	429		392	393	394	395	396	397

cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned
16	16	16	16	16	16	17	17	17	17	17
500, 400	500, 400	900, 700, 500, 400, 2000	900, 700, 500, 400, 200	900, 800, 500 4000, 350	900, 800, 500, 400, 350	500, 400, 150	500, 400, 150	900, 700, 500, 400, 300, 150	900, 700, 500, 400, 300, 150	500, 400
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06
37	37	37	37	37	37	37	37	37	37	37
20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
12	10	10	12	12	13	12	12	12	12	12
5.5	7.5	7.5	5.5	5.5	4.5	5.5	5.5	5.5	5.5	5.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	2	2	2	2	2	2	2	2	2	2
398	399	400	401	402	403	375	376	377	378	379

cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned		cleaned	cleaned	cleaned
17	17	17	17	17	17	17	17	18	18	18
500, 400	cannot be determined	cannot be determined	900, 700, 550, 450, 400	900, 700, 550, 450, 400	550, 450, 400	550, 450, 400		1300, 800, 500, 400, 200, 150	1300, 800, 500, 400, 200, 150	900, 800, 500, 400,
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	23-Jan-06	27-Jan-06	27-Jan-06	27-Jan-06
37	37	37	37	37	37	37		37	37	37
							X			
20.5	20.5	20.5	20.5	20.5	20.5	20.5		20.5	20.5	20.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5	0.5	0.5
12	14	14	14	14	14	14		14	14	14
5.5	3.5	3.5	3.5	3.5	3.5	3.5		3.5	3.5	3.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5	0.5	0.5
2	2	2	2	2	2	2		2	2	2
380	381	382	383	384	385	386		356	388	45

cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned
18	18	18	18	18	18	18	18	18	19	19
900, 800, 500, 400,	1300, 900, 800, 500, 300, 250, 200, 150	1300, 900, 800, 500, 300, 250, 200, 150	1400, 1300, 900, 800, 500, 300, 250, 200, 150	14000, 1400, 900, 800, 500, 300, 250, 200, 150	1400, 1300, 900, 800, 500, 300, 250,	1400, 1300, 900, 800, 500, 300, 250,	1300, 900, 800, 500, 450, 400, 150	1300, 900, 800, 500, 450, 400, 300, 150	500, 400, 150	500, 400, 150
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
27-Jan-06	27-Jan-06	27-Jan-06	27-Jan-06	27-Jan-06	27-Jan-06	27-Jan-06	27-Jan-06	27-Jan-06	14-Mar-06	14-Mar-06
37	37	37	37	37	37	37	37	37	37	37
20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
14	14	14	14	14	15	14	14	14	12	12
3.5	3.5	3.5	3.5	3.5	2.5	3.5	3.5	3.5	5.5	5.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	2	2	2	2	2	2	2	2	2	2
56	357	370	410	411	392	393	372	373	434	435

cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned
19	19	19	19	19	19	19	19	20	20	20
500, 400, 350	500, 400, 350	500, 400, 350	500, 400, 350	900, 800, 500, 450, 350, 200	900, 800, 500, 450, 350, 200	900, 800, 500, 450, 350, 200	900, 800, 500, 450, 350, 200	500, 450, 400	500, 450, 400	900, 800, 550, 450, 400
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
14-Mar-06	14-Mar-06	14-Mar-06	14-Mar-06	14-Mar-06	14-Mar-06	14-Mar-06	14-Mar-06	15-Mar-06	15-Mar-06	15-Mar-06
37	37	37	37	37	37	37	37	37	37	37
20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
12	12	12	12	14	14	14	14	14	14	14
5.5	5.5	5.5	5.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	2	2	2	2	2	2	2	2	2	2
448	451	452	255	440	441	442	443	406	407	446

cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned	cleaned
20	20	20	20	20	20	20	20	20	21	21
900, 800, 550, 450, 400	900, 800, 550, 450, 400	900, 800, 550, 450, 400	550, 450, 400	550, 450, 400	900, 800, 500, 450	900, 800, 500, 450	Cannot be determine	Cannot be determine	Cannot be determine	Cannot be determine
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
15-Mar-06	15-Mar-06	15-Mar-06	15-Mar-06	15-Mar-06	15-Mar-06	15-Mar-06	15-Mar-06	15-Mar-06	15-Mar-06	15-Mar-06
37	37	37	37	37	37	37	37	37	37	37
20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
14	14	14	14	14	14	14	14	14	14	14
3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	2	2	2	2	2	2	2	2	2	2
447	315	316	436	437	392	393	372	373	384	385

cleaned	cleaned	cleaned	cleaned
21	21	21	21
Cannot be determine	Cannot be determine	Cannot be determine	Cannot be determine
2.5	2.5	2.5	2.5
15-Mar-06	15-Mar-06	15-Mar-06	15-Mar-06
37	37	37	37
20.5	20.5	20.5	20.5
0.5	0.5	0.5	0.5
14	14	14	14
3.5	3.5	3.5	3.5
0.5	0.5	0.5	0.5
2	2	2	2
372	373	408	409

Table 9 Wetland comparison

SEASON	BAND SIZE (BP)	DIFFERENCES	GROUPS
Fall	1400, 1300, **1000**, 900, **850,** 800, 500, 450, 400, **300,** 250, 200, 150	**1400, 850, 550, 300**	13
Winter	1300, 900, 800, **550,** 500, 450, 400, 250, 200, 150.		10
Spring	1300, 900, 800, 500, 450, 400, 250, 200, 150,		9
Summer	1400, 1300, 900, 800, 500, 450, 400, 200, 150		9
Depth			
0-9 in	1400, 1300, 900, 800, **850, 650,** 550, 500, 450, 400, **350**, 300, **250**, 200, 150	**850, 700, 650, 600, 350, 250**	15
10-27 in	1400, 1300, 1000, 900, 800, **700**, 550, 500, 450, 400, 300, 200, 150		13
28-45 in	1400, 1300, 1000, 900, 800, **600,** 550, 500, 450, 400, 300, 200, 150.		13
Wetland			
Valle G	**1400,** 1300, **1000**, 900, 850, 800, 700, **600**, 550, 500, 450, 400, 300, 250, 200, 150,	**1400, 1000, 600,**	16
WPAFB	1300, 900, 850, 800, 700, 550, 500, 450, 400, 300, 250, 200, 150		13

Overall seasonal comparison

Fall	Winter	Spring	Summer	Difference	Similarities
1400	1300	1300	1400	1400	1300
1300	900	900	1300	850	900
1000	800	800	900	550	800
900	550	500	800	300	500
850	500	450	500		450
800	450	400	450		400
500	400	250	400		200
450	250	200	200		150
400	200	150	150		
300	150				
250					
200					
150					

Overall depth comparison

0 to 9	10 to 27	28 to 45	Difference	Similarities
1400	1400	1400	550	**1400**
1300	1300	1300	300	**1300**
900	900	900		**900**
800	800	800		**800**
500	550	500		**500**
450	500	450		**450**
400	450	400		**400**
250	400	250		**250**
200	300	200		**200**
150	250	150		**150**
	200			
	150			

Wetland comparison

Valle Greene	WPAFB	Difference	Similarities
1400	1300	1400	**1300**
1300	900	1000	**900**
1000	800	850	**800**
900	500	550	**500**
850	450	300	**450**
800	400		**400**
550	250		**250**
500	200		**200**
450	150		**150**
400			
300			
250			
200			
150			

Across section 1

Base Pair Core 1-1	Base Pair Core 2-1	Base Pair Core 4-1	Base Pair Core 5-1	Base Pair Core 6-1	Base Pair Core 7-1	Base Pair Core 8-1	Base Pair Core 9-1	Difference	Similarities
500	900	800	1300	500	1400	1300	900	1400	1300
450	800	500	900	450	1300	900	800		900
450	500	450	800	400	900	800	500		800
	450	400	500		800	500	450		500
		200	450		500	450	300		450
		150	400		450	250	250		400
			250		400	200			200
			200		200				150
			150		150				

Across section 2

Base Pair Core 1-2	Base Pair Core 2-2	Base Pair Core 4-2	Base Pair Core 5-2	Base Pair Core 6-2	Base Pair Core 7-2	Base Pair Core 8-2	Base Pair Core 9-2	Similarities
900	1300	900	1300	500	1300	500	1400	500
800	900	800	900	450	900	450	900	450
500	800	500	800	400	800	400	800	400
450	500	450	500		500	200	500	
400	450	400	450		450	150	450	
	400		400		400		300	
	200		250		200		250	
	150		200		150			
			150					

Across section 3

Base Pair Core 1-3	Base Pair Core 2-3	Base Pair Core 4-3	Base Pair Core 5-3	Base Pair Core 6-3	Base Pair Core 7-3	Base Pair Core 8-3	Base Pair Core 9-3	Similarities
900	500	1300	500	500	900	500	1400	500
800	450	900	450	450	800	450	1000	450
500	400	800	400	400	500	400	900	400
450		500			450	200	850	
400		450			400	150	550	
		400			200		500	
		250			150		450	
		200						
		150						

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Vita

Captain G. K. Joseph graduated from Jose Antonio Remon Cantera High School in Panama City, Republic of Panama. He entered the United States Air Force in 1987 as a Power Production specialist and was assigned to Cheyenne Mountain AFS in Colorado Springs, Colorado. He was later transferred to Andersen AFB where he was promoted to SSgt in 1993. In 1994 he was transfer to Cheyenne Mountain AFS where he was selected for the Bootstrap program. He entered undergraduate studies at the University of Colorado at Colorado Springs, where he graduated with a Bachelor of Arts degree in Biology in May 1996. He was commissioned in 1997 as a Second Lieutenant after completion of Officer Candidate School in Quantico, Virginia. He then attended The Basic School (TBS) and Infantry Officer Course (IOC) in 1998. His first assignment was as a Rifle platoon commander at 3rd Battalion 7th Marines in Twenty-nine Palms, California. During that tour he was promoted to First Lieutenant and held the billets of Weapons platoon commander and Executive officer. In 2001 Captain Joseph was transferred to 3rd Force Service Support Group in Okinawa, Japan as Plans officer G4. He was then assigned as Headquarters' and Services Battalion Operations officer and later selected to command Headquarters' company in June 2002. In June 2004, he entered the Graduate School of Engineering and Management, Air Force Institute of Technology. Upon graduation, he will be assigned to 2nd Marine Logistic Group in Camp Lejeune, North Carolina as the environmental officer.

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14. ABSTRACT
The diversity of microbial communities in wetlands has not been fully measured. These communities may offer tools to naturally remediate sites polluted with chlorinated compounds, Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) are genomic techniques that are capable of rapidly characterizing bacteria communities and can provide valuable information about the diversity of microbial communities. In this research, the bacterial community profile of a constructed wetland receiving PCE-contaminated ground water at Wright-Patterson Air Force Base (WPAFB) in Dayton Ohio was compared to a naturally-occurring and non-polluted wetland in Beavercreek, Ohio. The top 45 inches of both wetlands were analyzed to identify prominent groups of bacterial community through base pair length analysis. Comparing relative microbial diversity across seasons the following was seen: fall 96.6%, winter 62.5%, spring 56.25%, and summer 69.32%. Between depths, the relativity was 79.37% in the first depth, 100% in the second depth, and 79.37% in the last depth. Valle Greene showed 100% relative diversity and WPAFB displayed 54.7% thus indicating RFLP as a viable procedure for identifying bacterial groups.

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